

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 July 2001 (05.07.2001)

PCT

(10) International Publication Number
WO 01/47880 A1

(51) International Patent Classification⁷: **C07D 207/34**,
A61K 31/40, C07D 401/04, A61P 29/00, A61K 31/402

(74) Agent: NAPOLI, James, J.; Marshall, O'Toole, Gerstein,
Murray & Borun, 6300 Sears Tower, 233 S. Wacker Drive,
Chicago, IL 60606 (US).

(21) International Application Number: PCT/US00/28496

(22) International Filing Date: 13 October 2000 (13.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/171,954 23 December 1999 (23.12.1999) US

(71) Applicant: **ICOS CORPORATION** [US/US]; 22021
20th Avenue, S.E., Bothell, WA 98021 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

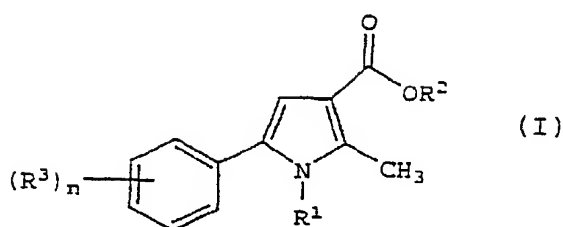
(72) Inventors: **MARTINS, Timothy, J.**; 3115 209th Street
SE, Bothell, WA 98021 (US). **FOWLER, Kerry, W.**; 747
N. 66th Street, Seattle, WA 98103 (US). **OLIVER, Amy**;
3312 189th Place SE, Bothell, WA 98021 (US). **HER-**
TEL, Carmen, C.; 14120 176th Place SE, Snohomish, WA
98290 (US).

Published:

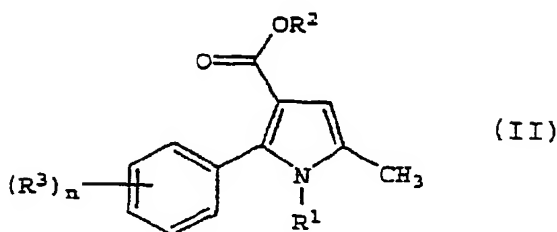
— With international search report.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: CYCLIC AMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS



(57) Abstract: Pyrrole compounds having structural formula (I) or (II) that are potent and selective inhibitors of PDE4, as well as methods of making the same, are disclosed. Use of the compounds in the treatment of inflammatory diseases and other diseases involving elevated levels of cytokines, as well as central nervous system (CNS) disorders, also is disclosed.



CYCLIC AMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of
provisional application Serial No. 60/171,954, filed
December 23, 1999.

FIELD OF INVENTION

10 The present invention relates to a series
of compounds that are potent and selective inhibi-
tors of cyclic adenosine 3',5'-monophosphate spe-
cific phosphodiesterase (cAMP-specific PDE). In
15 particular, the present invention relates to a se-
ries of novel pyrrole compounds that are useful for
inhibiting the function of cAMP-specific PDE, in
particular, PDE4, as well as methods of making the
same, pharmaceutical compositions containing the
20 same, and their use as therapeutic agents, for
example, in treating inflammatory diseases and other
diseases involving elevated levels of cytokines and
proinflammatory mediators.

25 BACKGROUND OF THE INVENTION

Chronic inflammation is a multi-factorial
disease complication characterized by activation of
multiple types of inflammatory cells, particularly
30 cells of lymphoid lineage (including T lymphocytes)
and myeloid lineage (including granulocytes, macro-
phages, and monocytes). Proinflammatory mediators,
including cytokines, such as tumor necrosis factor

- 2 -

(TNF) and interleukin-1 (IL-1), are produced by these activated cells. Accordingly, an agent that suppresses the activation of these cells, or their production of proinflammatory cytokines, would be
5 useful in the therapeutic treatment of inflammatory diseases and other diseases involving elevated levels of cytokines.

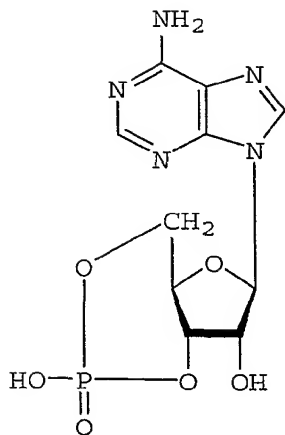
Cyclic adenosine monophosphate (cAMP) is a second messenger that mediates the biologic re-
10 sponses of cells to a wide range of extracellular stimuli. When the appropriate agonist binds to specific cell surface receptors, adenylate cyclase is activated to convert adenosine triphosphate (ATP) to cAMP. It is theorized that the agonist induced
15 actions of cAMP within the cell are mediated predominantly by the action of cAMP-dependent protein kinases. The intracellular actions of cAMP are terminated by either a transport of the nucleotide to the outside of the cell, or by enzymatic cleavage
20 by cyclic nucleotide phosphodiesterases (PDEs), which hydrolyze the 3'-phosphodiester bond to form 5'-adenosine monophosphate (5'-AMP). 5'-AMP is an inactive metabolite. The structures of cAMP and 5'-AMP are illustrated below.

25

- 3 -

5

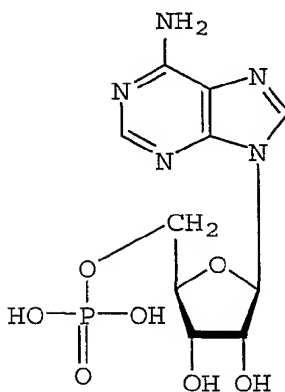
10



cAMP

15

20



25

5'-AMP

Elevated levels of cAMP in human myeloid and lymphoid lineage cells are associated with the suppression of cell activation. The intracellular enzyme family of PDEs, therefore, regulates the level of cAMP in cells. PDE4 is a predominant PDE isotype in these cells, and is a major contributor to cAMP degradation. Accordingly, the inhibition of

- 4 -

PDE function would prevent the conversion of cAMP to the inactive metabolite 5'-AMP and, consequently, maintain higher cAMP levels, and, accordingly, suppress cell activation (see Beavo et al., "Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action," Wiley and Sons, Chichester, pp. 3-14, (1990)); Torphy et al., *Drug News and Perspectives*, 6, pp. 203-214 (1993); Giembycz et al., *Clin. Exp. Allergy*, 22, pp. 337-344 (1992)).

10 In particular, PDE4 inhibitors, such as rolipram, have been shown to inhibit production of TNF α and partially inhibit IL-1 β release by monocytes (see Semmler et al., *Int. J. Immunopharmacol.*, 15, pp. 409-413, (1993); Molnar-Kimber et al., *Mediators of Inflammation*, 1, pp. 411-417, (1992)).

15 PDE4 inhibitors also have been shown to inhibit the production of superoxide radicals from human polymorphonuclear leukocytes (see Verghese et al., *J. Mol. Cell. Cardiol.*, 21 (Suppl. 2), S61 (1989);

20 Nielson et al., *J. Allergy Immunol.*, 86, pp. 801-808, (1990)); to inhibit the release of vasoactive amines and prostanoids from human basophils (see Peachell et al., *J. Immunol.*, 148, pp. 2503-2510, (1992)); to inhibit respiratory bursts in eosinophils (see Dent et al., *J. Pharmacol.*, 103, pp. 1339-1346, (1991)); and to inhibit the activation of

25 human T-lymphocytes (see Robicsek et al., *Biochem. Pharmacol.*, 42, pp. 869-877, (1991)).

Inflammatory cell activation and excessive

30 or unregulated cytokine (e.g., TNF α and IL-1 β) production are implicated in allergic, autoimmune, and inflammatory diseases and disorders, such as rheuma-

- 5 -

toid arthritis, osteoarthritis, gouty arthritis, spondylitis, thyroid associated ophthalmopathy, Behcet's disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, 5 toxic shock syndrome, asthma, chronic bronchitis, adult respiratory distress syndrome, chronic pulmonary inflammatory disease, such as chronic obstructive pulmonary disease, silicosis, pulmonary sarcoidosis, reperfusion injury of the myocardium, brain, 10 and extremities, fibrosis, cystic fibrosis, keloid formation, scar formation, atherosclerosis, transplant rejection disorders, such as graft vs. host reaction and allograft rejection, chronic glomerulonephritis, lupus, inflammatory bowel disease, such 15 as Crohn's disease and ulcerative colitis, proliferative lymphocyte diseases, such as leukemia, and inflammatory dermatoses, such as atopic dermatitis, psoriasis, and urticaria.

Other conditions characterized by elevated 20 cytokine levels include brain injury due to moderate trauma (see Dhillon et al., *J. Neurotrauma*, 12, pp. 1035-1043 (1995); Suttorp et al., *J. Clin. Invest.*, 91, pp. 1421-1428 (1993)), cardiomyopathies, such as congestive heart failure (see Bristow et al., *Circulation*, 97, pp. 1340-1341 (1998)), cachexia, cachexia 25 secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), ARC (AIDS related complex), fever myalgias due to infection, cerebral malaria, osteoporosis and 30 bone resorption diseases, keloid formation, scar tissue formation, and pyrexia.

In particular, TNF α has been identified as having a role with respect to human acquired immune

- 6 -

deficiency syndrome (AIDS). AIDS results from the infection of T-lymphocytes with Human Immunodeficiency Virus (HIV). Although HIV also infects and is maintained in myeloid lineage cells, TNF has been shown to upregulate HIV infection in T-lymphocytic and monocytic cells (see Poli et al., *Proc. Natl. Acad. Sci. USA*, 87, pp. 782-785, (1990)).

Several properties of TNF α , such as stimulation of collagenases, stimulation of angiogenesis *in vivo*, stimulation of bone resorption, and an ability to increase the adherence of tumor cells to endothelium, are consistent with a role for TNF in the development and metastatic spread of cancer in the host. TNF α recently has been directly implicated in the promotion of growth and metastasis of tumor cells (see Orosz et al., *J. Exp. Med.*, 177, pp. 1391-1398, (1993)).

PDE4 has a wide tissue distribution. There are at least four genes for PDE4 of which multiple transcripts from any given gene can yield several different proteins that share identical catalytic sites. The amino acid identity between the four possible catalytic sites is greater than 85%. Their shared sensitivity to inhibitors and their kinetic similarity reflect the functional aspect of this level of amino acid identity. It is theorized that the role of these alternatively expressed PDE4 proteins allows a mechanism by which a cell can differentially localize these enzymes intracellularly and/or regulate the catalytic efficiency via post translational modification. Any given cell type that expresses the PDE4 enzyme typi-

- 7 -

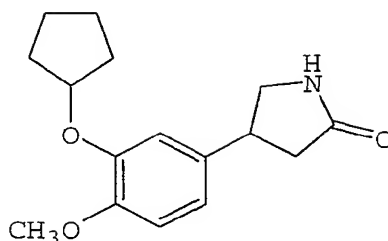
cally expresses more than one of the four possible genes encoding these proteins.

Investigators have shown considerable interest in the use of PDE4 inhibitors as anti-inflammatory agents. Early evidence indicates that PDE4 inhibition has beneficial effects on a variety of inflammatory cells such as monocytes, macrophages, T-cells of the Th-1 lineage, and granulocytes. The synthesis and/or release of many pro-inflammatory mediators, such as cytokines, lipid mediators, superoxide, and biogenic amines, such as histamine, have been attenuated in these cells by the action of PDE4 inhibitors. The PDE4 inhibitors also affect other cellular functions including T-cell proliferation, granulocyte transmigration in response to chemotoxic substances, and integrity of endothelial cell junctions within the vasculature.

The design, synthesis, and screening of various PDE4 inhibitors have been reported. Methylxanthines, such as caffeine and theophylline, were the first PDE inhibitors discovered, but these compounds are nonselective with respect to which PDE is inhibited. The drug rolipram, an antidepressant agent, was one of the first reported specific PDE4 inhibitors. Rolipram, having the following structural formula, has a reported 50% Inhibitory Concentration (IC_{50}) of about 200 nM (nanomolar) with respect to inhibiting recombinant human PDE4.

- 8 -

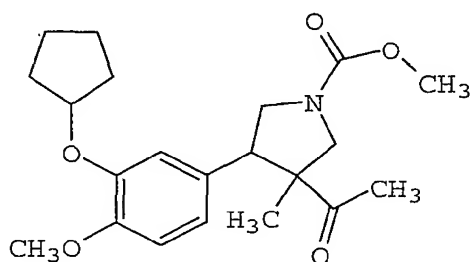
5



Rolipram

10 Investigators have continued to search for
PDE4 inhibitors that are more selective with respect
to inhibiting PDE4, that have a lower IC₅₀ than
rolipram, and that avoid the undesirable central
nervous system (CNS) side effects, such as retching,
15 vomiting, and sedation, associated with the adminis-
tration of rolipram. One class of compounds is dis-
closed in Feldman et al. U.S. Patent No. 5,665,754.
The compounds disclosed therein are substituted
pyrrolidines having a structure similar to rolipram.
20 One particular compound, having the following struc-
tural formula, has an IC₅₀ with respect to human
recombinant PDE4 of about 2 nM. Inasmuch as a fa-
vorable separation of emetic side effect from effi-
cacy was observed, these compounds did not exhibit a
25 reduction in undesirable CNS effects.

30



- 9 -

In addition, several companies are now undertaking clinical trials of other PDE4 inhibitors. However, problems relating to efficacy and adverse side effects, such as emesis and central nervous system disturbances, remain unsolved.

Accordingly, compounds that selectively inhibit PDE4, and that reduce or eliminate the adverse CNS side effects associated with prior PDE4 inhibitors, would be useful in the treatment of allergic and inflammatory diseases, and other diseases associated with excessive or unregulated production of cytokines, such as TNF. In addition, selective PDE4 inhibitors would be useful in the treatment of diseases that are associated with elevated cAMP levels or PDE4 function in a particular target tissue.

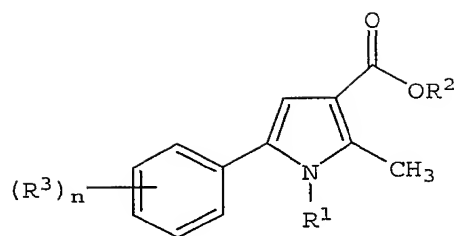
SUMMARY OF THE INVENTION

The present invention is directed to potent and selective PDE4 inhibitors useful in treatment of diseases and conditions where inhibition of PDE4 activity is considered beneficial. The present PDE4 inhibitors unexpectedly reduce or eliminate the adverse CNS side effects associated with prior PDE4 inhibitors.

In particular, the present invention is directed to pyrrole compounds having the structural formula (I) or (II):

- 10 -

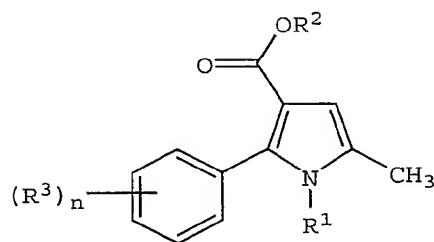
5



(I)

10

15



(II)

wherein R^1 is selected from the group consisting of optionally substituted aryl, alkyl, cycloalkyl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)alkyl$, NR^4R^5 , $C(=O)NR^5R^6$, $C(=O)Oalkyl$, CO_2H , $OC(=O)alkyl$, nitro, halo, alkylthio, $SO_2(alkyl)$, SO_3H , and haloalkyl;

R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

- 11 -

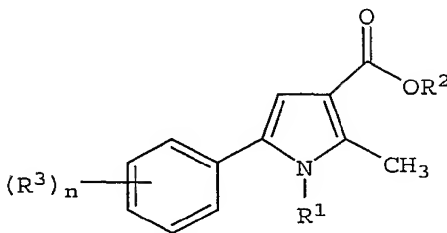
The present invention also is directed to pharmaceutical compositions containing one or more of the compounds of structural formula (I) or (II), to use of the compounds and compositions containing the compounds in the treatment of a disease or disorder, and to methods of preparing the compounds and intermediates involved in the synthesis of the compounds of structural formula (I) and (II).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 contains plots of TNF α concentration in serum (pg/mL) vs. concentration of PDE4 inhibitors.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

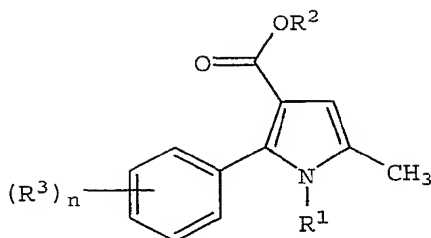
The present invention is directed to compounds having a structural formula (I) or (II):



(I)

- 12 -

5



(II)

10 wherein

R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

15 R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$ alkyl, NR^4R^5 , $C(=O)NR^5R^6$, $C(=O)O$ alkyl, CO_2H , $OC(=O)$ alkyl, nitro, halo, alkylthio, SO_2 (alkyl), SO_3H , and haloalkyl;

R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

25 As used herein, the term "alkyl," alone or in combination, is defined to include straight chain and branched chain, and bridged, saturated hydrocarbon groups containing one to 16 carbon atoms. The term "lower alkyl" is defined herein as an alkyl group having one through six carbon atoms (C_1 - C_6).
 30 Examples of lower alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, isobutyl, n-butyl, neopentyl, -hexyl, and the like.

- 13 -

The term "bridged alkyl" is defined herein as a C₆-C₁₆ bicyclic or polycyclic hydrocarbon group, for example, norboryl, adamantyl, bicyclo[2.2.2]-octyl, bicyclo[2.2.1]heptyl, bicyclo[3.2.1]octyl, or
5 decahydronaphthyl.

The term "cycloalkyl" is defined herein to include cyclic C₃-C₇ hydrocarbon groups. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclohexyl, and cyclo-
10 pentyl.

The term "haloalkyl" is defined herein as an alkyl group substituted with one or more halo substituents, either fluoro, chloro, bromo, iodo, or combinations thereof. Similarly, "halocycloalkyl" is defined as a cycloalkyl group having one or more
15 halo substituents.

The term "aryl," alone or in combination, is defined herein as a monocyclic or polycyclic aromatic group, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl, that can
20 be unsubstituted or substituted, for example, with one or more, and in particular one to three, substituents selected from halo, alkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkyl-
25 sulfinyl, and alkylsulfonyl. Exemplary aryl groups include phenyl, naphthyl, tetrahydronaphthyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 2-methylphenyl, 4-methoxyphenyl, 3-trifluoromethyl-
30 phenyl, 4-nitrophenyl, and the like.

The term "heteroaryl" is defined herein as a monocyclic or bicyclic ring system containing one or two aromatic rings and containing at least one

- 14 -

nitrogen, oxygen, or sulfur atom in an aromatic ring, and which can be unsubstituted or substituted, for example, with one or more, and in particular one to three, substituents, like halo, alkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkylsulfinyl, and alkylsulfonyl. Examples of heteroaryl groups include thienyl, furyl, pyridyl, oxazolyl, quinolyl, isoquinolyl, indolyl, triazolyl, isothiazolyl, isoxazolyl, imidazolyl, benzothiazolyl, pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.

The term "aralkyl" is defined herein as a previously defined alkyl group, wherein one of the hydrogen atoms is replaced by an aryl group as defined herein, for example, a phenyl group optionally having one or more substituents, for example, halo, alkyl, alkoxy, and the like. An example of an aralkyl group is a benzyl group.

The term "alkaryl" is defined herein as a previously defined aryl group, wherein one of the hydrogen atoms is replaced by an alkyl, cycloalkyl, haloalkyl, or halocycloalkyl group.

The term "heteroaralkyl" and "heteroalkaryl" are defined similarly as the terms "aralkyl" and "alkaryl," however, the aryl group is replaced by a heteroaryl group as previously defined.

The term "heterocycle" or "heterocyclic ring" is defined as a 5- or 6-membered nonaromatic ring having one or more heteroatoms selected from oxygen, nitrogen, and sulfur present in the ring. Nonlimiting examples include tetrahydrofuran, piperidine, piperazine, sulfolane, morpholine, tetra-

- 15 -

hydropyran, dioxane, and the like. A "carbocyclic ring" is similarly defined, but the ring contains solely carbon atoms.

5 The term "halogen" or "halo" is defined herein to include fluorine, chlorine, bromine, and iodine.

The terms "alkoxy," "aryloxy," and "aralkoxy" are defined as -OR, wherein R is alkyl, aryl, and aralkyl, respectively.

10 The term "alkoxyalkyl" is defined as an alkoxy group appended to an alkyl group. The terms "aryloxyalkyl" and "aralkoxyalkyl" are similarly defined as an aryloxy or aralkoxy group appended to an alkyl group.

15 The term "hydroxy" is defined as -OH.

The term "hydroxyalkyl" is defined as a hydroxy group appended to an alkyl group.

The term "amino" is defined as -NH₂.

20 The term "alkylamino" is defined as -NR₂ wherein at least one R is alkyl and the second R is alkyl or hydrogen.

The term "acylamino" is defined as RC(=O)NH, wherein R is alkyl or aryl.

The term "nitro" is defined as -NO₂.

25 The term "alkylthio" is defined as -SR, where R is alkyl.

The term "alkylsulfinyl" is defined as R-S(O)₂, where R is alkyl.

30 The term "alkylsulfonyl" is defined as R-S(O₂), where R is alkyl.

In preferred embodiments, R¹ is selected from the group consisting of aryl, heteroaryl,

- 16 -

alkaryl, aralkyl, and cycloalkyl; R^2 is selected from the group consisting of hydrogen and lower alkyl.

In most preferred embodiments, R^1 is aryl, heteroaryl, cycloalkyl, or aralkyl, optionally substituted with one or more of NR^4R^5 , halo, alkyl, $C(=O)alkyl$, $SO_2(alkyl)$, $C(=O)NR^4R^5$, SO_3H , NO_2 , CO_2H , $C(=O)Oalkyl$, and trifluoromethyl; R^2 is hydrogen or $C_{1-2}alkyl$, and R^3 is hydrogen. R^1 typically is phenyl or pyridyl, optionally substituted, and R^2 is hydrogen or ethyl.

The present invention includes all possible stereoisomers and geometric isomers of compounds of structural formulae (I) and (II), and includes not only racemic compounds but also the optically active isomers as well. When a compound of structural formulae (I) and (II) is desired as a single enantiomer, it can be obtained either by resolution of the final product or by stereospecific synthesis from either isomerically pure starting material or use of a chiral auxiliary reagent, for example, see Z. Ma et al., *Tetrahedron: Asymmetry*, 8(6), pages 883-888 (1997). Resolution of the final product, an intermediate, or a starting material can be achieved by any suitable method known in the art. Additionally, in situations where tautomers of the compounds of structural formulae (I) and (II) are possible, the present invention is intended to include all tautomeric forms of the compounds. As demonstrated hereafter, specific stereoisomers exhibit an exceptional ability to inhibit PDE4 without manifesting the adverse CNS side effects typically associated with PDE4 inhibitors.

- 17 -

Compounds of structural formula (I) and (II) which contain acidic moieties can form pharmaceutically acceptable salts with suitable cations. Suitable pharmaceutically acceptable cations include
5 alkali metal (e.g., sodium or potassium) and alkaline earth metal (e.g., calcium or magnesium) cations. The pharmaceutically acceptable salts of the compounds of structural formula (I) and (II), which contain a basic center, are acid addition salts
10 formed with pharmaceutically acceptable acids. Examples include the hydrochloride, hydrobromide, sulfate or bisulfate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, fumarate, maleate, lactate, citrate, tartrate, gluconate, methane-
15 sulfonate, benzenesulphonate, and p-toluenesulphonate salts. In light of the foregoing, any reference to compounds of the present invention appearing herein is intended to include compounds of structural formula (I) and (II), as well as pharmaceutically acceptable salts and solvates thereof.
20

The compounds of the present invention can be therapeutically administered as the neat chemical, but it is preferable to administer compounds of structural formula (I) and (II) as a pharmaceutical
25 composition or formulation. Accordingly, the present invention further provides for pharmaceutical formulations comprising a compound of structural formula (I) and/or (II), or pharmaceutically acceptable salts thereof, together with one or more pharmaceutically acceptable carriers and, optionally,
30 other therapeutic and/or prophylactic ingredients. The carriers are "acceptable" in the sense of being

- 18 -

compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

In particular, a selective PDE4 inhibitor of the present invention is useful alone or in combination with a second antiinflammatory therapeutic agent, for example, a therapeutic agent targeting TNF α , such as ENBREL® or REMICADE®, which have utility in treating rheumatoid arthritis. Likewise, therapeutic utility of IL-1 antagonism has also been shown in animal models for rheumatoid arthritis. Thus, it is envisioned that IL-1 antagonism, in combination with PDE4 inhibition, which attenuates TNF α , would be efficacious.

The present PDE4 inhibitors are useful in the treatment of a variety of allergic, autoimmune, and inflammatory diseases.

The term "treatment" includes preventing, lowering, stopping, or reversing the progression of severity of the condition or symptoms being treated. As such, the term "treatment" includes both medical therapeutic and/or prophylactic administration, as appropriate.

In particular, inflammation is a localized, protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (i.e., sequester) both the injurious agent and the injured tissue. The term "inflammatory disease," as used herein, means any disease in which an excessive or unregulated inflammatory response leads to excessive inflammatory symptoms, host tissue damage, or loss of tissue function. Additionally, the term "autoimmune disease," as used herein, means any group of disorders in

- 19 -

which tissue injury is associated with humoral or cell-mediated responses to the body's own constituents. The term "allergic disease," as used herein, means any symptoms, tissue damage, or loss of tissue function resulting from allergy. The term "arthritis disease," as used herein, means any of a large family of diseases that are characterized by inflammatory lesions of the joints attributable to a variety of etiologies. The term "dermatitis," as used herein, means any of a large family of diseases of the skin that are characterized by inflammation of the skin attributable to a variety of etiologies. The term "transplant rejection," as used herein, means any immune reaction directed against grafted tissue (including organ and cell (e.g., bone marrow)), characterized by a loss of function of the grafted and surrounding tissues, pain, swelling, leukocytosis and thrombocytopenia.

The present invention also provides a method of modulating cAMP levels in a mammal, as well as a method of treating diseases characterized by elevated cytokine levels.

The term "cytokine," as used herein, means any secreted polypeptide that affects the functions of other cells, and that modulates interactions between cells in the immune or inflammatory response. Cytokines include, but are not limited to monokines, lymphokines, and chemokines regardless of which cells produce them. For instance, a monokine is generally referred to as being produced and secreted by a monocyte, however, many other cells produce monokines, such as natural killer cells, fibroblasts, basophils, neutrophils, endothelial

- 20 -

cells, brain astrocytes, bone marrow stromal cells, epidermal keratinocytes, and B-lymphocytes. Lymphokines are generally referred to as being produced by lymphocyte cells. Examples of cytokines include,
5 but are not limited to, interleukin-1 (IL-1), interleukin-6 (IL-6), Tumor Necrosis Factor alpha (TNF α), and Tumor Necrosis Factor beta (TNF β).

The present invention further provides a method of reducing TNF levels in a mammal, which
10 comprises administering an effective amount of a compound of structural formula (II) to the mammal. The term "reducing TNF levels," as used herein, means either:

a) decreasing excessive *in vivo* TNF
15 levels in a mammal to normal levels or below normal levels by inhibition of the *in vivo* release of TNF by all cells, including but not limited to monocytes or macrophages; or

b) inducing a down-regulation, at the
20 translational or transcription level, of excessive *in vivo* TNF levels in a mammal to normal levels or below normal levels; or

c) inducing a down-regulation, by inhibition of the direct synthesis of TNF as a postrans-
25 lational event.

Moreover, the compounds of the present invention are useful in suppressing inflammatory cell activation. The term "inflammatory cell activation," as used herein, means the induction by a
30 stimulus (including, but not limited to, cytokines, antigens or auto-antibodies) of a proliferative cellular response, the production of soluble mediators (including but not limited to cytokines, oxygen

- 21 -

radicals, enzymes, prostanoids, or vasoactive amines), or cell surface expression of new or increased numbers of mediators (including, but not limited to, major histocompatibility antigens or cell adhesion molecules) in inflammatory cells (including but not limited to monocytes, macrophages, T lymphocytes, B lymphocytes, granulocytes, polymorphonuclear leukocytes, mast cells, basophils, eosinophils, dendritic cells, and endothelial cells). It will be appreciated by persons skilled in the art that the activation of one or a combination of these phenotypes in these cells can contribute to the initiation, perpetuation, or exacerbation of an inflammatory condition.

The compounds of the present invention also are useful in causing airway smooth muscle relaxation, bronchodilation, and prevention of bronchoconstriction.

The compounds of the present invention, therefore, are useful in treating such diseases as arthritic diseases (such as rheumatoid arthritis), osteoarthritis, gouty arthritis, spondylitis, thyroid-associated ophthalmopathy, Behcet disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, toxic shock syndrome, asthma, chronic bronchitis, allergic rhinitis, allergic conjunctivitis, vernal conjunctivitis, eosinophilic granuloma, adult (acute) respiratory distress syndrome (ARDS), chronic pulmonary inflammatory disease (such as chronic obstructive pulmonary disease), silicosis, pulmonary sarcoidosis, reperfusion injury of the myocardium, brain or extremities, brain or spinal cord injury due to minor

- 22 -

trauma, fibrosis including cystic fibrosis, keloid formation, scar tissue formation, atherosclerosis, autoimmune diseases, such as systemic lupus erythematosus (SLE) and transplant rejection disorders (e.g., graft vs. host (GvH) reaction and allo-graft rejection), chronic glomerulonephritis, inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, proliferative lymphocytic diseases, such as leukemias (e.g. chronic lymphocytic leukemia; CLL) (see Mentz et al., *Blood* 88, pp. 2172-2182 (1996)), and inflammatory dermatoses, such as atopic dermatitis, psoriasis, or urticaria.

Other examples of such diseases or related conditions include cardiomyopathies, such as congestive heart failure, pyrexia, cachexia, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), ARC (AIDS-related complex), cerebral malaria, osteoporosis and bone resorption diseases, and fever and myalgias due to infection. In addition, the compounds of the present invention are useful in the treatment of diabetes insipidus and central nervous system disorders, such as depression and multiinfarct dementia.

Compounds of the present invention also have utility outside of that typically known as therapeutic. For example, the present compounds can function as organ transplant preservatives (see Pinsky et al., *J. Clin. Invest.*, 92, pp. 2994-3002 (1993)) as well.

Selective PDE4 inhibitors also can be useful in the treatment of diabetes insipidus (*Kidney Int.*, 37, p. 362, (1990); *Kidney Int.*, 35, p.

- 23 -

494, (1989)) and central nervous system disorders, such as multiinfarct dementia (Nicholson, *Psychopharmacology*, 101, p. 147 (1990)), depression (Eckman et al., *Curr. Ther. Res.*, 43, p. 291 (1988)), anxiety and stress responses (*Neuropharmacology*, 38, p. 1831 (1991)), cerebral ischemia (*Eur. J. Pharmacol.*, 272, p. 107 (1995)), tardive dyskinesia (*J. Clin. Pharmacol.*, 16, p. 304 (1976)), Parkinson's disease (see *Neurology*, 25, p. 722 (1975); *Clin. Exp. Pharmacol, Physiol.*, 26, p. 421 (1999)), and premenstrual syndrome. With respect to depression, PDE4-selective inhibitors show efficacy in a variety of animal models of depression such as the "behavioral despair" or Porsolt tests (*Eur. J. Pharmacol.*, 47, p. 379 (1978); *Eur. J. Pharmacol.*, 57, p. 431 (1979); *Antidepressants: neurochemical, behavioral and clinical perspectives*, Enna, Malick, and Richelson, eds., Raven Press, p. 121 (1981)), and the "tail suspension test" (*Psychopharmacology*, 85, p. 367 (1985)). Recent research findings show that chronic *in vivo* treatment by a variety of antidepressants increase the brain-derived expression of PDE4 (*J. Neuroscience*, 19, p. 610 (1999)). Therefore, a selective PDE4 inhibitor can be used alone or in conjunction with a second therapeutic agent in a treatment for the four major classes of antidepressants: electroconvulsive procedures, monoamine oxidase inhibitors, and selective reuptake inhibitors of serotonin or norepinephrine. Selective PDE4 inhibitors also can be useful in applications that modulate bronchodilatory activity via direct action

- 24 -

on bronchial smooth muscle cells for the treatment of asthma.

Compounds and pharmaceutical compositions suitable for use in the present invention include those wherein the active ingredient is administered in an effective amount to achieve its intended purpose. More specifically, a "therapeutically effective amount" means an amount effective to prevent development of, or to alleviate the existing symptoms of, the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

A "therapeutically effective dose" refers to that amount of the compound that results in achieving the desired effect. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD_{50} and ED_{50} . Compounds which exhibit high therapeutic indices are preferred. The data obtained from such data can be used in formulating a dosage range for use in humans. The dosage of such compounds preferably lies within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed, and the route of administration utilized.

- 25 -

The exact formulation, route of administration, and dosage can be chosen by the individual physician in view of the patient's condition. Dosage amount and interval can be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the therapeutic effects.

As appreciated by persons skilled in the art, reference herein to treatment extends to prophylaxis, as well as to treatment of established diseases or symptoms. It is further appreciated that the amount of a compound of the invention required for use in treatment varies with the nature of the condition being treated, and with the age and the condition of the patient, and is ultimately determined by the attendant physician or veterinarian. In general, however, doses employed for adult human treatment typically are in the range of 0.001 mg/kg to about 100 mg/kg per day. The desired dose can be conveniently administered in a single dose, or as multiple doses administered at appropriate intervals, for example as two, three, four or more subdoses per day. In practice, the physician determines the actual dosing regimen which is most suitable for an individual patient, and the dosage varies with the age, weight, and response of the particular patient. The above dosages are exemplary of the average case, but there can be individual instances in which higher or lower dosages are merited, and such are within the scope of the present invention.

Formulations of the present invention can be administered in a standard manner for the treatment of the indicated diseases, such as orally,

- 26 -

parenterally, transmucosally (e.g., sublingually or via buccal administration), topically, transdermally, rectally, via inhalation (e.g., nasal or deep lung inhalation). Parenteral administration includes, but is not limited to intravenous, intra-arterial, intraperitoneal, subcutaneous, intramuscular, intrathecal, and intraarticular. Parenteral administration also can be accomplished using a high pressure technique, like POWDERJECT™.

10 For buccal administration, the composition can be in the form of tablets or lozenges formulated in conventional manner. For example, tablets and capsules for oral administration can contain conventional excipients such as binding agents (for example, syrup, accacia, gelatin, sorbitol, tragacanth, 15 mucilage of starch or polyvinylpyrrolidone), fillers (for example, lactose, sugar, microcrystalline, cellulose, maize-starch, calcium phosphate or sorbitol), lubricants (for example, magnesium, stearate, stearic acid, talc, polyethylene glycol or silica), 20 disintegrants (for example, potato starch or sodium starch glycollate), or wetting agents (for example, sodium lauryl sulfate). The tablets can be coated according to methods well known in the art.

25 Alternatively, the compounds of the present invention can be incorporated into oral liquid preparations such as aqueous or oily suspensions, solutions, emulsions, syrups, or elixirs, for example. Moreover, formulations containing these compounds can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can contain conventional additives, such as suspending agents, such as 30

- 27 -

sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, hydroxypropylmethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats; 5 emulsifying agents, such as lecithin, sorbitan mono-oleate, or acacia; nonaqueous vehicles (which can include edible oils), such as almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol; and preservatives, such as methyl or 10 propyl p-hydroxybenzoate and sorbic acid.

Such preparations also can be formulated as suppositories, e.g., containing conventional suppository bases, such as cocoa butter or other glycerides. Compositions for inhalation typically 15 can be provided in the form of a solution, suspension, or emulsion that can be administered as a dry powder or in the form of an aerosol using a conventional propellant, such as dichlorodifluoromethane or trichlorofluoromethane. Typical topical and 20 transdermal formulations comprise conventional aqueous or nonaqueous vehicles, such as eye drops, creams, ointments, lotions, and pastes, or are in the form of a medicated plaster, patch, or membrane.

Additionally, compositions of the present 25 invention can be formulated for parenteral administration by injection or continuous infusion. Formulations for injection can be in the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulation agents, such as 30 suspending, stabilizing, and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable

- 28 -

vehicle (e.g., sterile, pyrogen-free water) before use.

A composition in accordance with the present invention also can be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Accordingly, the compounds of the invention can be formulated with suitable polymeric or hydrophobic materials (e.g., an emulsion in an acceptable oil), ion exchange resins, or as sparingly soluble derivatives (e.g., a sparingly soluble salt).

For veterinary use, a compound of formula (I) or (II), or nontoxic salts thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal.

Thus, the invention provides in a further aspect a pharmaceutical composition comprising a compound of the formula (I) or (II), together with a pharmaceutically acceptable diluent or carrier therefor. There is further provided by the present invention a process of preparing a pharmaceutical composition comprising a compound of formula (I) or (II), which process comprises mixing a compound of formula (I) or (II), together with a pharmaceutically acceptable diluent or carrier therefor.

Specific, nonlimiting examples of compounds of structural formula (I) and (II) are pro-

- 29 -

vided below, the synthesis of which were performed in accordance with the procedures set forth below.

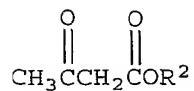
Generally, compounds of structural formula (I) and (II) can be prepared according to the following synthetic scheme. In the scheme described below, it is understood in the art that protecting groups can be employed where necessary in accordance with general principles of synthetic chemistry. These protecting groups are removed in the final steps of the synthesis under basic, acidic, or hydrogenolytic conditions which are readily apparent to those skilled in the art. By employing appropriate manipulation and protection of any chemical functionalities, synthesis of compounds of structural formula (I) and (II) not specifically set forth herein can be accomplished by methods analogous to the schemes set forth below.

Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification. All reactions and chromatography fractions were analyzed by thin-layer chromatography on 250-mm silica gel plates, visualized with UV (ultraviolet) light and I₂ (iodine) stain. Flash column chromatography was carried out using Biotage 40M silica gel (230-400 mesh). Products and intermediates were purified by flash chromatography or reverse-phase HPLC.

As illustrated below, the compounds of general structural formula (I) are prepared by reacting a compound having the formula,

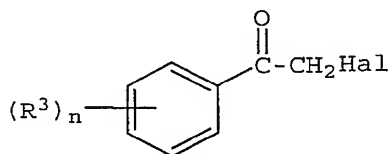
- 30 -

5



with sodium metal to generate a carbanion, then reacting the carbanion with a compound having the formula

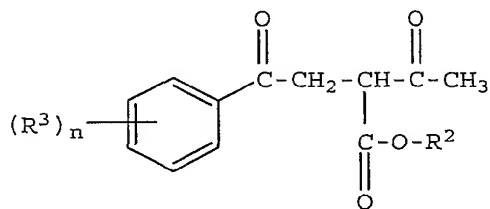
10



15

wherein Hal is a halo, typically bromo, to generate an intermediate compound having the formula

20



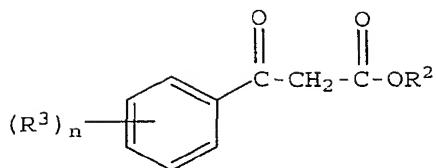
25

This intermediate compound then is reacted with a primary amine having the formula R^1NH_2 to provide a compound of structural formula (I).

30

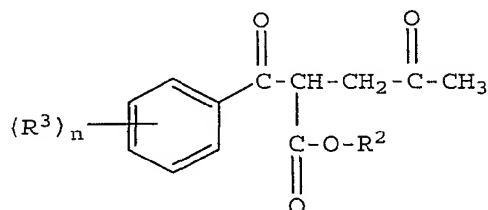
A compound of structural formula (II) is prepared by reacting a compound having the formula

- 31 -



5

with chloroacetone to generate an intermediate compound having the formula



10

15

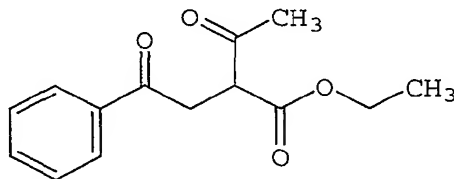
This intermediate compound then is reacted with a primary amine having the formula $R^1\text{NH}_2$ to provide a compound of structural formula (II).

20

Intermediate 1

Preparation of 3-Oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester

25



30

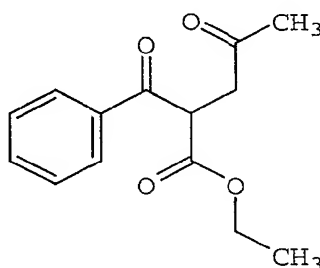
35 Sodium metal (60mmol, 1.4g) was suspended in 80mL (milliliter) toluene under nitrogen. Ethyl acetoacetate (88mmol (millimole), 11.4g) was added drop-

- 32 -

wise, and the resulting mixture was stirred until all sodium was consumed. The reaction mixture then was cooled in an ice bath (0-5°C) and 2-bromoaceto-phenone (60mmol, 12g) in 140mL toluene was added dropwise. The reaction was stirred overnight with gradual warming to room temperature. Analysis of the reaction mixture by GC and TLC showed that the reaction was complete. The reaction mixture then was washed with 200mL water, then the organic and aqueous layers were separated. The organic layer was dried over MgSO₄ (magnesium sulfate). The toluene was removed from the organic layer under vacuum to provide a yellow oil which was purified by Kugelrohr distillation (b.p. 135-145°C at 0.35 Torr.).

Intermediate 2

Preparation of 2-Benzoyl-4-oxo-pentanoic acid ethyl ester



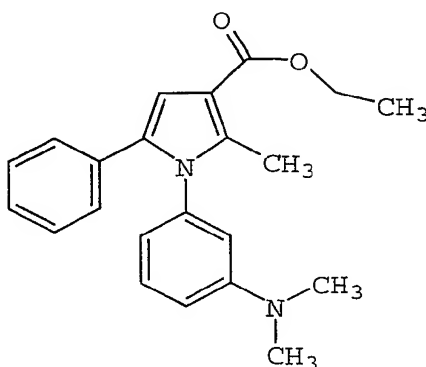
Potassium carbonate (200mmol, 27.6g) was added to a solution of ethyl benzoylacetate (100mmol, 19.2g) and potassium iodide (20mmol, 3.3g) in dry acetone (100mL). The resulting solution was refluxed, and chloroacetone (110mmol, 10.7g) was added dropwise. Analysis of the reaction mixture by GC/MS indicated

- 33 -

the presence of Intermediate 2. Solid material was filtered from the reaction mixture, then washed with 200mL acetone. The resulting filtrate was concentrated under vacuum, redissolved in ether, then washed three times with water (100mL). The organic layer was separated from the aqueous layer, and dried over MgSO_4 . The solids were separated, and the organic solvents removed under vacuum to yield a yellow/orange oil. The crude oil was purified by Kugelrohr distillation (b.p. 175-185 at 0.65 Torr.).

Example 1

Preparation of 1-(3-Dimethylaminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester



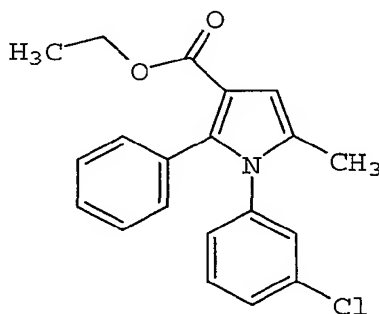
N,N-dimethyl-1,3-phenylenediamine dihydrochloride (5mmol, 1.0g), triethylamine (10mmol, 1.4mL), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol (25mL), then heated under reflux (12 hrs). The solvents were removed under vacuum, the resulting oil was redissolved in methylene chloride, then applied to a silica column. The purified product was collected as a translucent oil. The named

- 34 -

compound was identified by ^1H -NMR (CDCl_3 , ppm): 1.28 t (3H), 2.35 s (3H), 2.77 s (6H), 4.2 q (2H), 6.3-6.7 m (4H), and 7.0-7.3 m (5H).

5 Example 2

Preparation of 1-(3-Chlorophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester



10

15

20

m-Chloroaniline (5mmol, 0.6g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. Upon completion of the reaction, the crude solid was recrystallized from ethanol to provide the named product (m.p. 93.2°C-94.6°C).

25

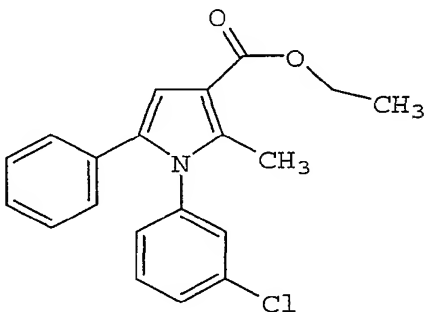
- 35 -

Example 3Preparation of 1-(3-Chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20

25

m-Chloroaniline (5mmol, 0.6g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. Upon completion of the reaction, the crude solid was recrystallized from ethanol to afford the desired product (m.p. 99.6°C-100.5°C).

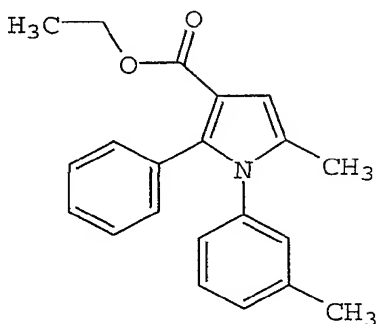
- 36 -

Example 4Preparation of 5-Methyl-2-phenyl-1-m-tolyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20 m-Toluidine (5mmol, 0.5g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting oil was resuspended in methylene chloride, then washed with water, 5% sodium bicarbonate, and brine. The organic layer was dried over NaSO₄ (sodium sulfate), the solids were separated, and the solvents evaporated under vacuum. The crude product was recrystallized from ethanol to yield the named product (m.p. 92.5°C-94.0°C).

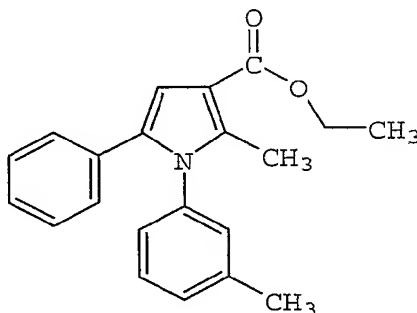
30

- 37 -

Example 5Preparation of 2-Methyl-5-phenyl-1-m-tolyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10



15

m-Toluidine (5mmol, 0.5g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting oil was resuspended in methylene chloride, then washed with water, 5% sodium bicarbonate, and brine. The organic layer was dried over NaSO₄, then the solids were separated and the solvents evaporated under vacuum. The crude product was recrystallized from ethanol to yield the named product (m.p. 103°C-104.5°C).

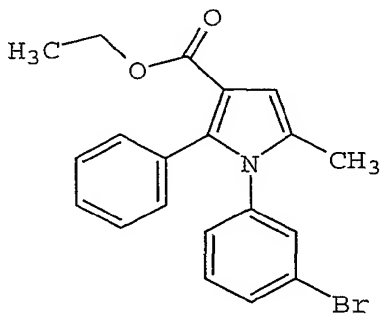
- 38 -

Example 6Preparation of 1-(3-Bromophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



m-Bromoaniline (5mmol, 0.9g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid were combined in ethanol, then heated under reflux. The resulting oil was resuspended in methylene chloride, then washed with water, 5% sodium bicarbonate and brine. The organic layer was dried over MgSO_4 , then the solids were separated and the solvents evaporated under vacuum. The crude product was recrystallized from ethanol to yield the named product (m.p. 117.5°C-119.2°C).

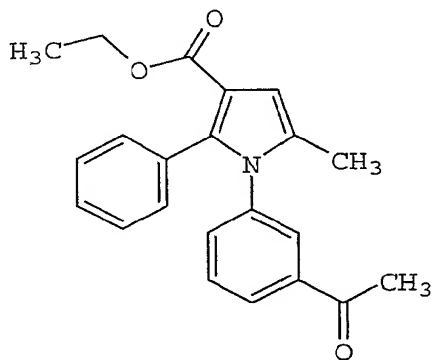
- 39 -

Example 7Preparation of 1-(3-Acetylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



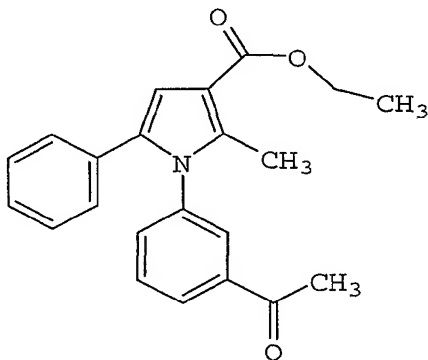
3'-Amino-acetophenone (5mmol, 0.7g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The residual oil was purified over silica and yielded the named product (m.p. 100°C-102°C).

25

Example 8Preparation of 1-(3-Acetylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

30

35

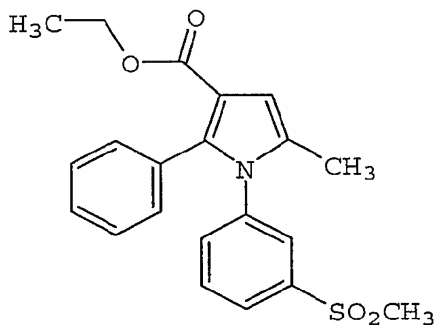


- 40 -

3'-Aminoacetophenone (5mmol, 0.7g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting oil was purified over silica and yielded the named product as a solid (m.p. 102°C-104°C).

Example 9

Preparation of 1-(3-Methanesulfonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester



3-Methylsulfonylaniline hydrochloride (5.8mmol, 1.2g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5.8mmol, 1.44g), triethylamine (5.8mmol, 0.61g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting oil was resuspended in chloroform, then washed with water, 5% sodium bicarbonate, and brine. The organic layer was dried over NaSO₄, then the solids were separated and solvents removed under vacuum. The crude material was purified over silica to yield the named product (m.p. 131°C-133°C).

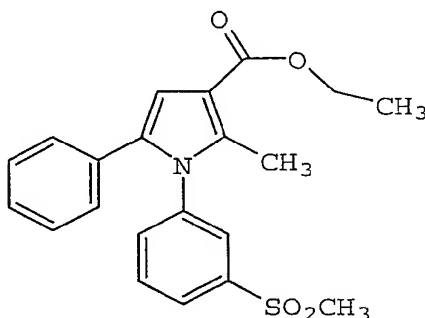
- 41 -

Example 10Preparation of 1-(3-Methanesulfonylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



3-Methylsulfonylaniline (1.4mmol, 0.3g), 3-oxo-2-(2-
20 oxo-2-phenyl-ethyl)-butyric acid ethyl ester
(1.4mmol, 0.35g), triethylamine (1.4mmol, 0.14g),
and tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The resulting oil was resus-
pended in chloroform, then washed with water, 5%
25 sodium bicarbonate, and brine. The organic layer
was dried over NaSO₄, then the solids were separated
and the solvents removed under vacuum. The crude
material was purified over silica gel to yield the
named product as an oil. The structure of the com-
30 pound was verified by ¹H-NMR (CDCl₃, ppm): 1.36 t
(3H), 2.45 s (3H), 2.82 s (3H), 4.3 q (2H), and 6.8-
7.2 m (9H).

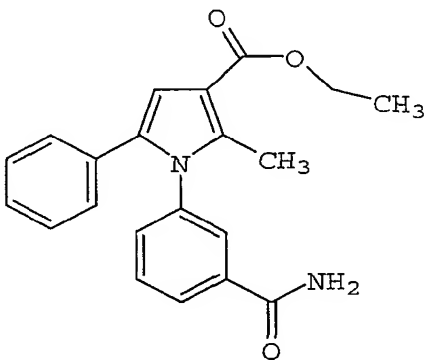
- 42 -

Example 11Preparation of 1-(3-Carbamoylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



3-Aminobenzamide (5mmol, 1.0g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting precipitate was filtered from the mixture, then washed with ethanol. The white solid product was used without further purification (m.p. 237.8°C-238.8°C).

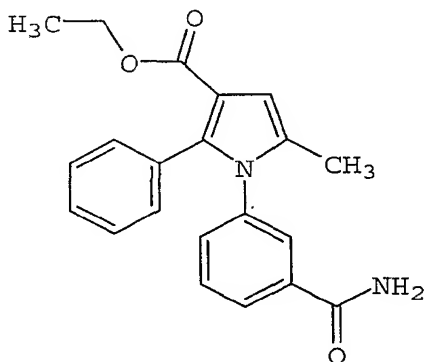
- 43 -

Example 12Preparation of 1-(3-Carbamoylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20

3-Aminobenzamide (5mmol, 1.0g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting precipitate was filtered from the reaction mixture, then washed with ethanol. The tan solid product was used without further purification (m.p. 208.9°C-210.3°C).

25

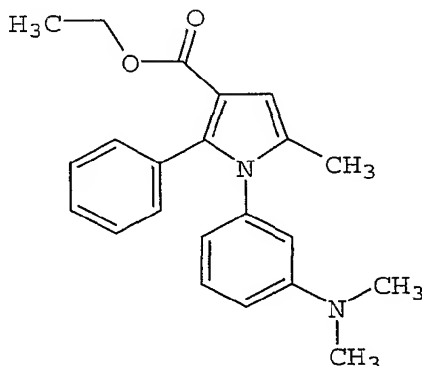
- 44 -

Example 13Preparation of 1-(3-Dimethylaminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20 N,N-Dimethyl-1,3-phenylenediamine dihydrochloride
(5mmol, 1.0g), 2-benzoyl-4-oxo-pentanoic acid ethyl
ester (5mmol, 1.2g), triethylamine (10mmol, 1.0g),
and tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The resulting oil was resus-
25 pended in methylene chloride, then washed with wa-
ter, 5% sodium bicarbonate, and brine. The organic
layer was dried over NaSO₄, then the solids were
separated and the solvents removed under vacuum.
The crude material was purified over silica gel and
30 yielded the named product (m.p. 111.7°C-114°C).

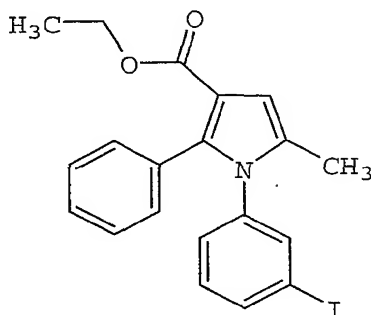
- 45 -

Example 14Preparation of 1-(3-Iodophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



m-Iodoaniline (5mmol, 1.1g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The residual oil was resuspended in methylene chloride, then washed with water, 5% sodium bicarbonate, and brine. The solids were separated, and the solvents removed under vacuum. The resulting solid was recrystallized in ethanol. A portion of the material did not dissolve, and was separated by filtration. Both the recrystallized and filtered material yielded the named product (m.p. 142.6°C-144°C).

30

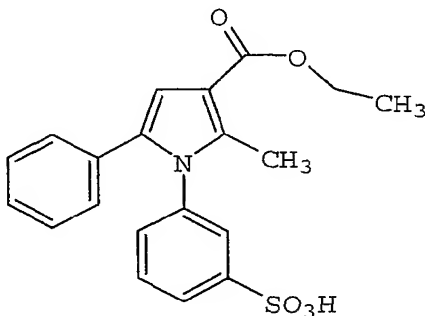
- 46 -

Example 15Preparation of 2-Methyl-5-phenyl-1-(3-sulfophenyl)-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20 Metanilic acid (5mmol, 0.9g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), sodium hydroxide (5mmol, 0.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The unreacted metanilic acid was

25 precipitated on ice and filtered from the reaction mixture. The filtrate was evaporated to yield an oil, which was purified over silica gel. After standing, the named product solidified as purple crystals (m.p. 94°C-96°C).

30

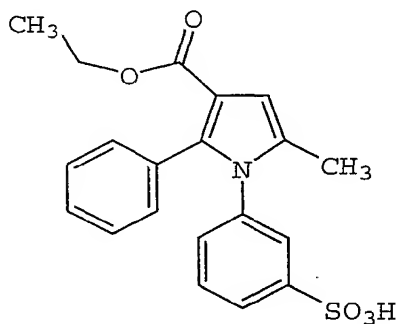
- 47 -

Example 16Preparation of 5-Methyl-2-phenyl-1-(3-sulfophenyl)-1H-pyrrole-3-carboxylic acid ethyl ester

5

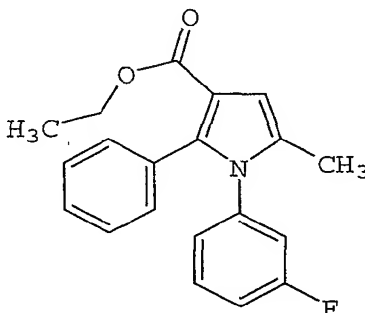
10

15



Metanilic acid (5mmol, 0.2g), 2-benzoyl-4-oxo-
20 pentanoic acid ethyl ester (5mmol, 1.2g), sodium
hydroxide (5mmol, 0.2g), and tosic acid (0.1g) were
combined in ethanol, then heated under reflux. The
resulting oil was treated with HCl (hydrochloric
acid) at 0°C to 5°C to precipitate the unreacted
25 metanilic acid. The remaining material was concen-
trated under vacuum and purified over silica gel.
The named product was collected as brown crystals
(m.p. 91°C-92.5°C).

- 48 -

Example 17Preparation of 1-(3-Fluorophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

m-Fluoroaniline (5mmol, 0.6g), 2-benzoyl-4-oxo-
20 pentanoic acid ethyl ester (5mmol, 1.2g), and tosic
acid (0.1g) were combined in ethanol, then heated
under reflux. The resulting oil was resuspended in
chloroform, then washed with water, 5% sodium bicar-
bonate, and brine. The organic layer was dried over
25 NaSO₄, then the solids filtered away and the solvents
removed under vacuum. The named product was recryst-
tallized from ethanol (m.p. 100°C-101°C).

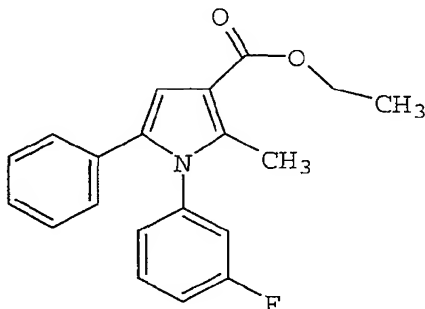
- 49 -

Example 18Preparation of 1-(3-Fluorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15

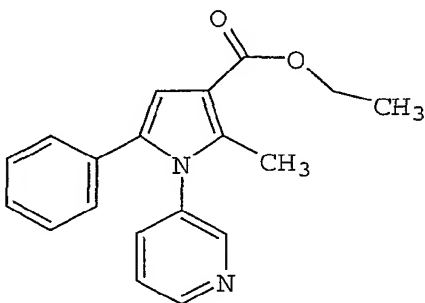


m-Fluoroaniline (5mmol, 0.6g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid were combined in ethanol, then heated under reflux. The resulting solid was resuspended in chloroform, then washed with water, 5% sodium bicarbonate, and brine. The organic layer was dried over NaSO₄, then the solids were separated and the solvents removed under vacuum. The named product was recrystallized from ethanol (m.p. 96.3°C-98.0°C).

Example 19Preparation of 2-Methyl-5-phenyl-1-pyridin-3-yl-1H-pyrrole-3-carboxylic acid ethyl ester

35

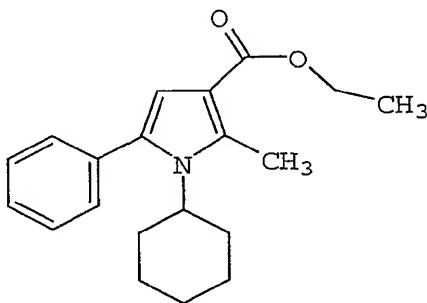
40



- 50 -

3-Aminopyridine (5mmol, 0.5g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g) and tosic acid (0.1g) were combined in ethanol, then
5 heated under reflux. The resulting oil was resuspended in ether, then washed with water and brine. The organic layer was dried over MgSO_4 , then the solids were separated and the solvents were removed under vacuum. The named product was purified over
10 silica gel and analyzed by ^1H -NMR (CDCl_3 , ppm): 1.35 t (3H), 2.40 s (3H), 4.3 q (2H), 6.78 s (1H), 7.03-7.39 m (7H), 8.4 sd (1H), and about 8.6 dd (1H).

- 51 -

Example 20Preparation of 1-Cyclohexyl-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

Cyclohexylamine (5mmol, 0.5g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g) and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting residue was resuspended in ether, then washed with water and brine. The organic layer was dried over MgSO_4 , and the solids were separated and the solvents removed under vacuum. Purification of the oil yielded the named product, $^1\text{H-NMR}$ (CDCl_3 , ppm): 1.0 bm (2H), 1.23 t (3H), 1.6-1.7 bm (8H), 2.66 s (3H), 4.16 q (2H), 6.39 s (1H), and 7.25 bm (5H).

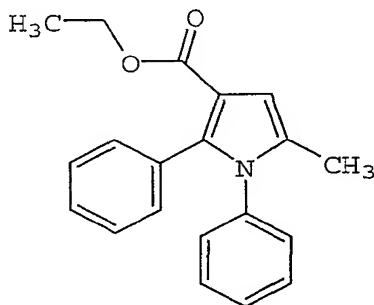
- 52 -

Example 21Preparation of 5-Methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20

Aniline (10mmol, 0.9g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (10mmol, 2.5g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The named product was recrystallized from ethanol (m.p. 130°C-131.7°C).

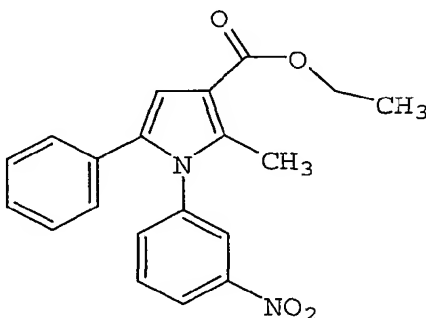
Example 22

25

Preparation of 2-Methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

30

35



40

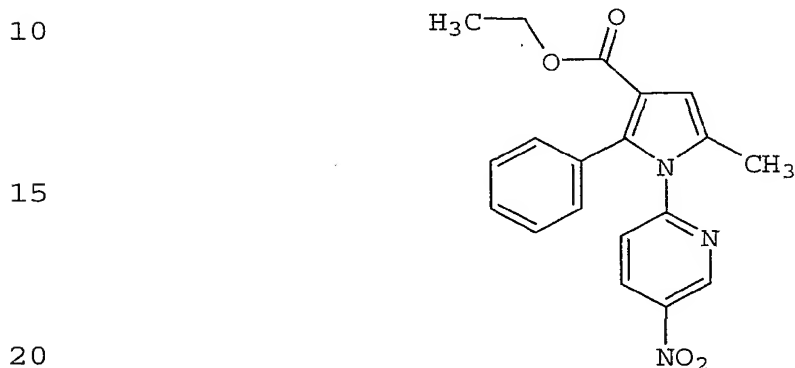
m-Nitroaniline (10mmol, 1.4g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (10mmol, 2.5g), and tosic acid (0.1g) were combined in etha-

- 53 -

nol, then heated under reflux. The named product was recrystallized from ethanol (m.p. 143°C-145°C).

Example 23

5 Preparation of 5-Methyl-1-(5-nitropyridin-2-yl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester



5-Nitro-2-aminopyridine (5mmol, 0.7g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting solid was triturated with chloroform, and the insoluble material filtered from the reaction mixture. The filtrate was purified over silica gel and yielded the named product (m.p. 146.6°C-148.6°C).

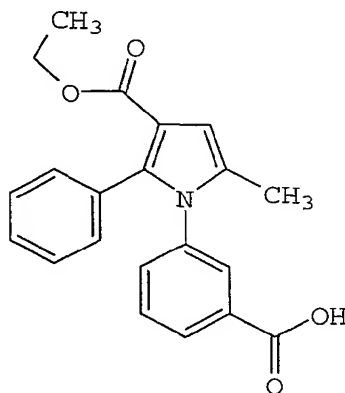
- 54 -

Example 24Preparation of 1-(3-Carboxyphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20 3-Aminobenzoic acid (10mmol, 1.4g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (10mmol, 2.5g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting oil was resuspended in chloroform-ether, then the insoluble material was

25 filtered from the reaction product. The soluble material was purified over silica gel which yielded the named product (m.p. 161.0°C-162.7°C).

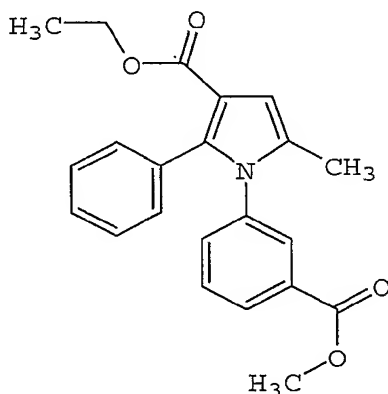
- 55 -

Example 25Preparation of 1-(3-Methoxycarbonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20

Methyl 3-aminobenzoate (10mmol, 1.4g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (10mmol, 2.5g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The named product was purified over silica gel and crystallized upon standing, ¹H-NMR (CDCl₃, ppm): 1.15 t (3H), 2.08 s (3H), 3.88 s (3H), 4.05 q (2H), 6.56 s (1H), 7.15-7.33 bm (7H), 7.79-7.97 bm (2H).

25

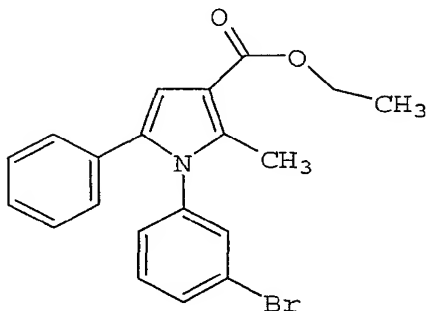
- 56 -

Example 26Preparation of 1-(3-Bromophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



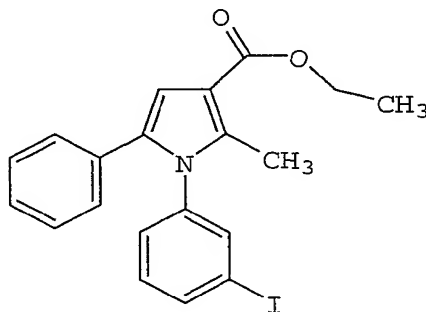
m-Bromoaniline (5mmol, 0.9g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The named product was recrystallized from ethanol (m.p. 111°C-112.7°C).

25 Example 27Preparation of 1-(3-Iodophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

30

35

40



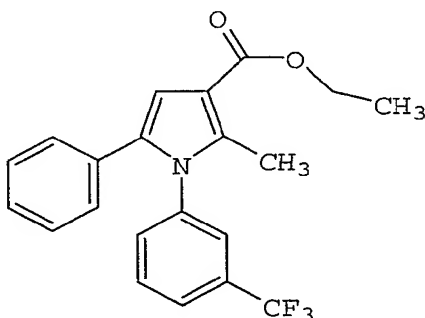
m-Iodoaniline (5mmol, 1.1g), 3-Oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol,

- 57 -

1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The named product was recrystallized in ethanol (m.p. 111°C-112.9°C).

5 Example 28

Preparation of 2-Methyl-5-phenyl-1-(3-trifluoromethylphenyl)-1H-pyrrole-3-carboxylic acid ethyl ester



m-Trifluoromethylaniline (5mmol, 0.8g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The crude material was resuspended in chloroform and washed with water, then with brine. The organic layer was dried over NaSO₄. The solids were separated and the solvents removed under vacuum. The resulting oil was purified over silica gel to yield the named product, ¹H-NMR (CDCl₃, ppm): 1.37 t (3H), 2.42 s (3H), 4.32 q (2H), 6.80 s (1H), 7.0-7.6 bm (9H).

35

- 58 -

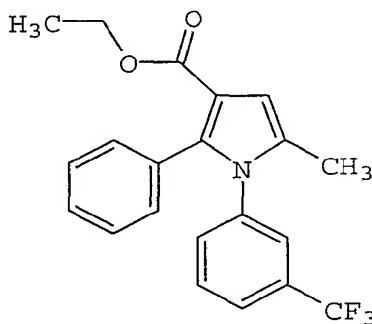
Example 29

Preparation of 5-Methyl-2-phenyl-1-(3-trifluoromethylphenyl)-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20 m-Trifluoromethylaniline (5mmol, 0.8g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The crude material was resuspended in chloroform, washed with water, then washed
25 with brine. The organic layer was dried over NaSO₄. The solids were separated and the solvents removed under vacuum. The resulting oil was purified over silica gel to yield the named product, ¹H-NMR (CDCl₃, ppm): 1.01 t (3H), 1.98 b (3H), 4.00 q (2H), 6.48 s
30 (1H), 7.00-7.33 bm (9H).

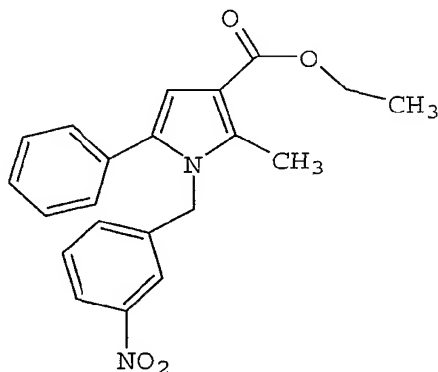
- 59 -

Example 30Preparation of 2-Methyl-1-(3-nitrobenzyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20 m-Nitrobenzylamine hydrochloride (3g) was suspended in an aqueous solution of saturated sodium bicarbonate (30mL), then extracted with chloroform. The organic layer was dried over MgSO_4 , the solids separated and solvents removed under vacuum to yield the

25 free base. The m-nitrobenzylamine (5mmol, 0.8g) prepared above, 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The residue was resuspended in ethyl ether,

30 and the benzylamine tosic acid salt was filtered from the mixture. The filtrate was concentrated under vacuum, and the resulting oil purified over silica to yield the named product, $^1\text{H-NMR}$ (CDCl_3 , ppm): 1.24 t (3H), 2.36 s (3H), 4.07-4.30 q (2H),

35 5.11 s (2H), 6.58 s (1H), 6.98-7.44 bm (7H), 7.69 bs (1H), 7.97 bd (1H).

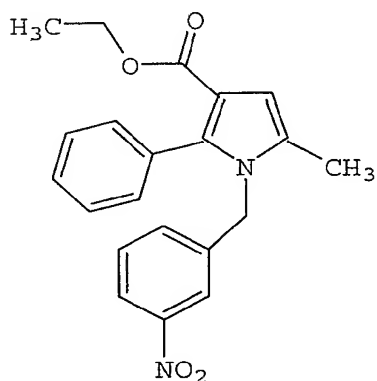
- 60 -

Example 31Preparation of 5-Methyl-1-(3-nitrobenzyl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20

m-Nitrobenzylamine hydrochloride (3g) was suspended in an aqueous solution of saturated sodium bicarbonate (30mL), and extracted with chloroform. The organic layer was dried over MgSO_4 , the solids saturated and solvents removed under vacuum to yield the free base. The m-nitrobenzylamine (5mmol, 0.8g) prepared above, 2-benzoyl-4-oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and tosic acid were combined in ethanol, then heated under reflux. The resulting residue was purified over silica gel to yield the named product, $^1\text{H-NMR}$ (CDCl_3 , ppm): 1.10 t (3H), 3.16 s (3H), 4.08 q (2H), 5.04 s (2H), 6.55 s (1H), 7.07-7.53 bm (7H), 7.72 bs (1H), 8.04 bd (1H).

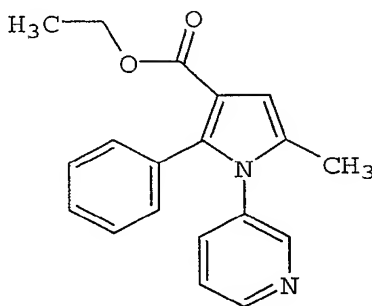
- 61 -

Example 32Preparation of 5-Methyl-2-phenyl-1-pyridin-3-yl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



3-Aminopyridine (5mmol, 0.5g), 2-benzoyl-4-oxo-
20 pentanoic acid ethyl ester (5mmol, 1.2g), and tosic
acid (0.1g) were combined in ethanol, then heated
under reflux. The resulting oil was purified over
silica, but still contained contaminants. This
material was triturated in hexane, and the solids
25 were separated by filtration. The solids were
washed with benzene two times to yield the named
product (m.p. 102°C-103°C).

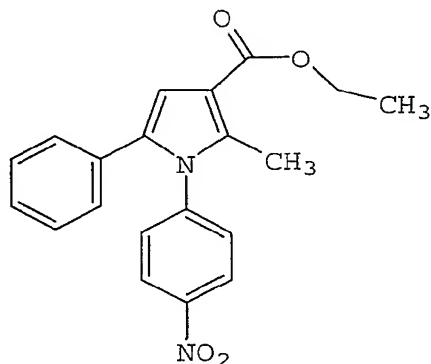
- 62 -

Example 33Preparation of 2-Methyl-1-(4-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

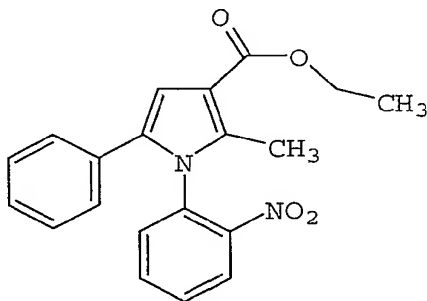
10

15

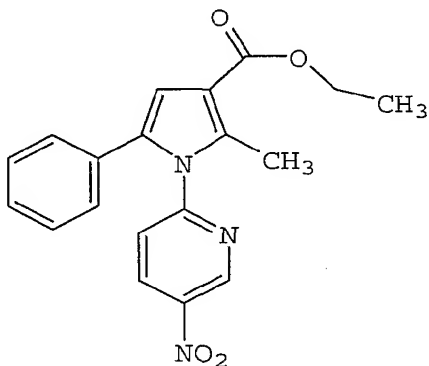


p-Nitroaniline (5mmol, 0.7g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.25g), and tosic acid were combined in ethanol, then heated under reflux. The reaction mixture was cooled on ice (0-5°C), and the precipitate filtered from the reaction mixture. ¹H-NMR analysis confirmed that the precipitate was the named product, (CDCl₃, ppm); 1.38 t (3H), 2.45 s (3H), 4.20 q (2H), 6.80 s (1H), 6.97-7.35 bm (7H), 8.25 d (2H).

- 63 -

Example 34Preparation of 2-Methyl-1-(2-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

o-Nitroaniline (15mmol, 2.1g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (15mmol, 3.7g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting oil was purified over three silica gel columns to yield the named product, ¹H-NMR (CDCl₃, ppm): 1.36 t (3H), 2.36 s (3H), 4.31 q (2H), 6.80 s (1H), 7.03-7.66 bm (6H), 7.68 dt (2H), 7.97 dd (1H).

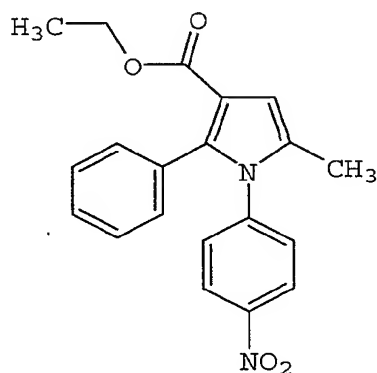
Example 35Preparation of 2-Methyl-1-(5-nitropyridin-2-yl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

- 64 -

5-Nitro-pyridin-2-ylamine (5mmol, 0.7g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting solid was triturated with chloroform, the filtrate was further purified over silica gel to yield the named product, ¹H-NMR (CDCl₃, ppm): 1.35 t (3H), 2.54 s (3H), 4.30 q (2H), 6.78 s (1H), 6.96-7.25 bm (6H), 8.33 dd (1H), 9.39 sd (1H).

Example 36

Preparation of 5-Methyl-1-(4-nitrophenyl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester



p-Nitroaniline (10mmol, 1.4g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (10mmol, 2.5g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The reaction mixture was filtered and the solids washed with ethanol. The collected material was determined to be the named product (m.p. 190°C-192°C).

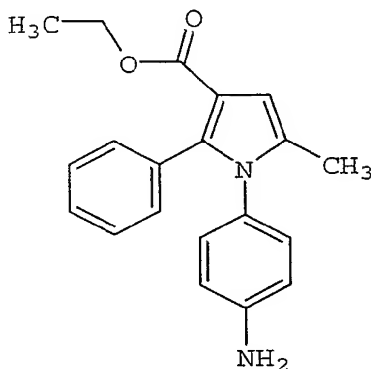
- 65 -

Example 37Preparation of 1-(4-Aminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20 5-Methyl-1-(4-nitrophenyl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester of Example 36 (4mmol, 1.5g) was dissolved in ethanol, and treated with 5% Pd/C (0.15g). The mixture was hydrogenated with H₂(g) at 45 psi. Upon completion of the reaction,
25 the mixture was filtered through Celite, and the solvents removed under vacuum. Analysis of the resulting solid showed it was the named product (m.p. 181°C-183°C).

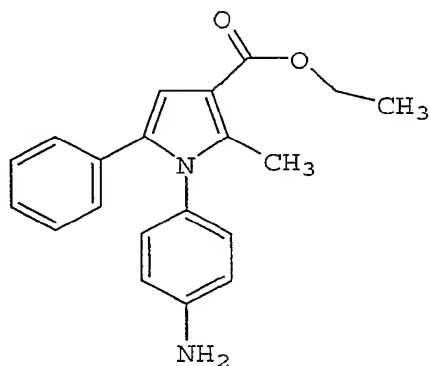
- 66 -

Example 38Preparation of 1-(4-Aminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



2-Methyl-1-(4-nitro-phenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester of Example 33 (2mmol, 0.8g) was dissolved 50% solution of ethyl acetate in ethanol, and treated with Pd/C (0.088g). The mixture was hydrogenated with H₂(g) at 45 psi. Upon completion of the reaction, the mixture was filtered through Celite and the solvents removed under vacuum. This material was purified over silica gel to yield the named product.

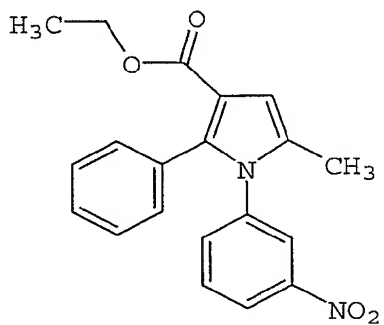
- 67 -

Example 391-(3-Nitrophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



The named product was purchased from
20 Maybridge Chemical Co., Ltd., Cornwall, UK, and used
without further purification.

25

30

35

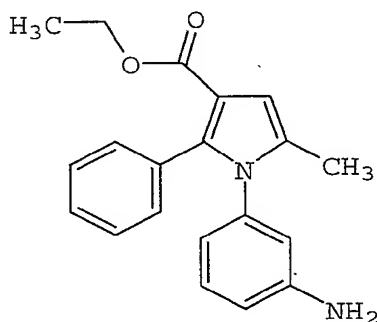
- 68 -

Example 40Preparation of 1-(3-Aminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



The compound of Example 39 (0.86mmol, 0.3g) was dissolved in methanol, and treated with nickel chloride (NiCl_2) and sodium borohydride (NaBH_4) (17.2mmol). The methanol was removed, and then the reaction mixture was resuspended in water, and next extracted with ethyl acetate. The reaction mixture was dried over MgSO_4 , filtered, and concentrated. The named product was purified over silica. ^1H -NMR (CDCl_3 , ppm): 1.37 t (3H), 2.41 s (3H), 3.73 s (2H), 4.31 q (2H), 6.43 s (1H), 6.54 d (1H), 6.67 dd (1H), 6.78 s (1H), 7.11-7.18 m (5H).

30

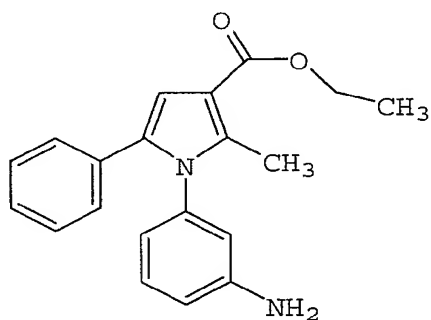
- 69 -

Example 41Preparation of 1-(3-Aminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20

The compound of Example 22 (0.86mmol, 0.3g) was dissolved in methanol and treated with NiCl_2 and NaBH_4 (17.2mmol) using the procedure set forth in Example 40. The named product was purified over silica (m.p. 142°C - 143°C).

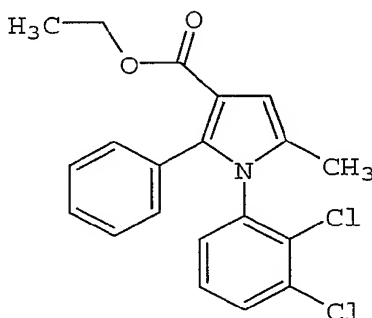
25

Example 421-(2,3-Dichlorophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

30

35

40

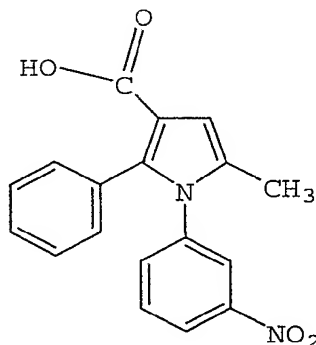


- 70 -

The named product was purchased from Maybridge Chemical Co., Ltd., and used without further purification.

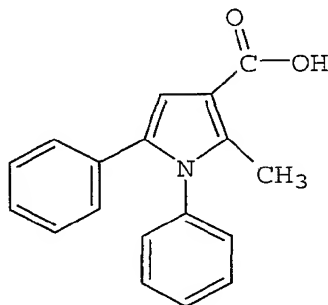
5 Example 43

Preparation of 5-Methyl-1-(3-nitrophenyl)-2-phenyl-1H-pyrrole-3-carboxylic acid

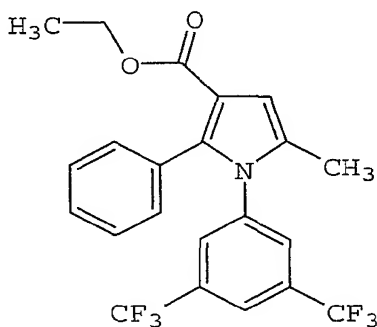


The compound of Example 39 (0.29mmol, 0.1g) was dissolved in a 50% dioxane and water, then treated with lithium hydroxide (LiOH, 0.87mmol, 0.0363g) and heated to at least 50°C. The solvents were removed and the base extracted with ethyl acetate. The aqueous layer was acidified to pH<2 and extracted with ethyl acetate. The organic layer was dried over MgSO₄, the solids filtered, and the solvents removed under vacuum. The named product was used without further purification. ¹H-NMR (CDCl₃, ppm): 2.48 s (3H), 6.88 s (1H), 7.03 dd (2H), 7.18 m (3H), 7.47 dd (1H), 7.6 t (1H), 8.08 st (1H), 8.27 dd (1H).

- 71 -

Example 44Preparation of 5-Methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid

The compound of Example 21 (0.33mmol, 0.1g) was hydrolyzed in the same manner as set forth in Example 43 to yield the named product, ¹H-NMR (CDCl₃, ppm): 2.44 s (3H), 6.87 s (1H), 7.06 m (2H), 7.15 m (5H), 7.40 m (3H).

Example 451-(3,5-Bis-trifluoromethylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

The named product was purchased from Maybridge Chemical Co., Ltd., and used without further purification.

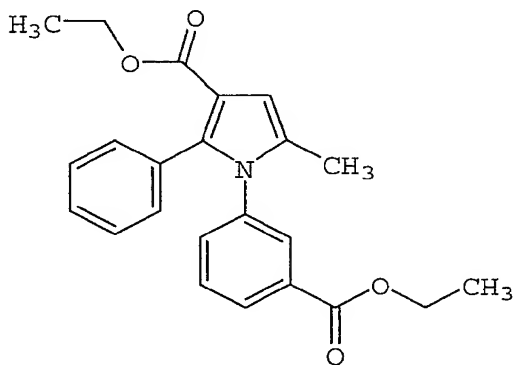
- 72 -

Example 46Preparation of 1-(3-Ethoxycarbonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15



20

This compound was formed in an identical manner as the compound of Example 25, except ethyl 3-aminobenzoate was used as a starting material. The named product had an m.p. of 108°C-110°C.

25

The compounds of structural formulae (I) and (II) were tested for an ability to inhibit PDE4. The ability of a compound to inhibit PDE4 activity is related to the IC_{50} value for the compound, i.e., the concentration of inhibitor required for 50% inhibition of enzyme activity. The IC_{50} value for compounds of structural formulae (I) and (II) were determined using human recombinant PDE4.

30

The compounds of the present invention typically exhibit an IC_{50} value against recombinant human PDE4 of less than about 100 μM , and preferably less than about 50 μM , and more preferably less than about 25 μM . The compounds of the present invention typically exhibit an IC_{50} value against recombinant human PDE4 of less than about 10 μM , and often less

35

- 73 -

than about 1 μM . To achieve the full advantage of the present invention, a present PDE4 inhibitor has an IC_{50} of about 0.005 μM to about 25 μM .

The IC_{50} values for the compounds were
5 determined from concentration-response curves typically using concentrations ranging from 0.1 pM to 500 μM . Tests against other PDE enzymes using standard methodology, as described in Loughney et al., *J. Biol. Chem.*, 271, pp. 796-806 (1996), also showed
10 that compounds of the present invention are highly selective for the cAMP-specific PDE4 enzyme.

In particular, a compound of the present invention, i.e., Example 22, has an IC_{50} vs. human recombinant PDE4B of 0.15 μM , but has an IC_{50} vs.
15 PDE1A of 35.5 μM , vs. PDE1B of 21.0 μM , vs. PDE1C of 21.8 μM , vs. PDE2 of 4.5 μM , vs. PDE3A of 89 μM , vs. PDE5 of 12.5 μM , and vs. PDE7 of 10.6 μM . This illustrates the selectivity of the present compound with respect to inhibiting PDE4.

20 The compounds of structural formulae (I) and (II) also were tested for an ability to reduce $\text{TNF}\alpha$ secretion in human peripheral blood lymphocytes. The ability to reduce $\text{TNF}\alpha$ secretion is related to the EC_{50} values (i.e., the effective concentration of the compound capable of inhibiting 50%
25 of the total $\text{TNF}\alpha$).

The compounds of the present invention typically exhibit an EC_{50} value of less than about 100 μM , and preferably less than about 50 μM , and
30 more preferably less than about 15 μM . The compounds of the present invention preferably exhibit a PBL/ $\text{TNF}\alpha$ EC_{50} value of less than about 10 μM , and often less than about 7 μM . To achieve the full

- 74 -

advantage of the present invention, a present PDE4 inhibitor has an EC_{50} value of about 1 μM to about 20 μM .

The production of recombinant human PDEs and the IC_{50} and EC_{50} determinations can be accomplished by well-known methods in the art. Exemplary methods are described as follows:

EXPRESSION OF HUMAN PDEs

10

Expression in Baculovirus-Infected *Spodoptera fugiperda* (Sf9) Cells

15

Baculovirus transfer plasmids were constructed using either pBlueBacIII (Invitrogen) or pFastBac (BRL-Gibco). The structure of all plasmids was verified by sequencing across the vector junctions and by fully sequencing all regions generated by PCR. Plasmid pBB-PDE1A3/6 contained the complete open reading frame of PDE1A3 (Loughney et al., *J. Biol. Chem.*, 271, pp. 796-806 (1996)) in pBlueBacIII. Plasmid Hcam3aBB contained the complete open reading frame of PDE1C3 (Loughney et al. (1996)) in pBlueBacIII. Plasmid pBB-PDE3A contained the complete open reading frame of PDE3A (Meacci et al., *Proc. Natl. Acad. Sci., USA*, 89, pp. 3721-3725 (1992)) in pBlueBacIII.

25

Recombinant virus stocks were produced using either the MaxBac system (Invitrogen) or the FastBac system (Gibco-BRL) according to the manufacturer's protocols. In both cases, expression of recombinant human PDEs in the resultant viruses was driven off the viral polyhedron promoter. When

30

- 75 -

using the MaxBac[®] system, virus was plaque purified twice in order to insure that no wild type (occ+) virus contaminated the preparation. Protein expression was carried out as follows. Sf9 cells were
5 grown at 27°C in Grace's Insect culture medium (Gibco-BRL) supplemented with 10% fetal bovine serum, 0.33% TC yeastolate, 0.33% lactalbumin hydrolysate, 4.2 mM NaHCO₃, 10 µg/mL gentamycin, 100 units/mL penicillin, and 100 µg/mL streptomycin.
10 Exponentially growing cells were infected at a multiplicity of approximately 2 to 3 virus particles per cell and incubated for 48 hours. Cells were collected by centrifugation, washed with non-supplemented Grace's medium, and quick-frozen for storage.
15 age.

Expression in *Saccharomyces cerevisiae* (Yeast)

Recombinant production of human PDE1B,
20 PDE2, PDE4A, PDE4B, PDE4C, PDE4D, PDE5, and PDE7 was carried out similarly to that described in Example 7 of U.S. Patent No. 5,702,936, incorporated herein by reference, except that the yeast transformation vector employed, which is derived from the basic
25 ADH2 plasmid described in Price et al., *Methods in Enzymology*, 185, pp. 308-318 (1990), incorporated yeast ADH2 promoter and terminator sequences and the *Saccharomyces cerevisiae* host was the protease-deficient strain BJ2-54 deposited on August 31, 1998
30 with the American Type Culture Collection, Manassas, Virginia, under accession number ATCC 74465. Transformed host cells were grown in 2X SC-leu medium, pH 6.2, with trace metals, and vitamins. After 24

- 76 -

hours, YEP medium-containing glycerol was added to a final concentration of 2X YET/3% glycerol. Approximately 24 hr later, cells were harvested, washed, and stored at -70°C.

5

CALMODULIN PURIFICATION

Calmodulin used for activation of the PDE1 enzymes was purified from bovine testes essentially as described by Dedman et al., *Methods in Enzymology*, 102, pp. 1-8 (1983) using the Pharmacia Phenyl-Sepharose[®] procedure.

IMMOBILIZATION OF CALMODULIN ON AGAROSE

15

Calmodulin was immobilized on BioRad Affi-Gel[®] 15 per manufacturer's instructions.

HUMAN PHOSPHODIESTERASE PREPARATIONS

20

Phosphodiesterase Activity Determinations

Phosphodiesterase activity of the preparations was determined as follows. PDE assays utilizing a charcoal separation technique were performed essentially as described in Loughney et al. (1996). In this assay, PDE activity converts [32P]cAMP or [32P]cGMP to the corresponding [32P]5'-AMP or [32P]5'-GMP in proportion to the amount of PDE activity present. The [32P]5'-AMP or [32P]5'-GMP then was quantitatively converted to free [32P]phosphate and unlabeled adenosine or guanosine by the action of snake venom 5'-nucleotidase. Hence, the amount

- 77 -

of [^{32}P]phosphate liberated is proportional to enzyme activity. The assay was performed at 30°C in a $100\text{ }\mu\text{L}$ reaction mixture containing (final concentrations) 40 mM Tris HCl ($\text{pH } 8.0$), $1\text{ }\mu\text{M}$ ZnSO_4 , 5 mM MgCl_2 , and 0.1 mg/mL bovine serum albumin (BSA). Alternatively, in assays assessing PDE1-specific activity, incubation mixtures further incorporated the use of 0.1 mM CaCl_2 and $10\text{ }\mu\text{g/mL}$ calmodulin. PDE enzyme was present in quantities that yield $<30\%$ total hydrolysis of substrate (linear assay conditions). The assay was initiated by addition of substrate (1 mM [^{32}P]cAMP or cGMP), and the mixture was incubated for 12 minutes. Seventy-five (75) μg of *Crotalus atrox* venom then was added, and the incubation was continued for 3 minutes (15 minutes total). The reaction was stopped by addition of $200\text{ }\mu\text{L}$ of activated charcoal (25 mg/mL suspension in 0.1 M NaH_2PO_4 , $\text{pH } 4$). After centrifugation (750 X g for 3 minutes) to sediment the charcoal, a sample of the supernatant was taken for radioactivity determination in a scintillation counter and the PDE activity was calculated.

Inhibitor analyses were performed similarly to the method described in Loughney et al., *J. Biol. Chem.*, 271, pp. 796-806 (1996), except both cGMP and cAMP were used, and substrate concentrations were kept below 32 nM , which is far below the K_m of the tested PDE5.

Purification of PDE1A3 from SF9 Cells

Cell pellets (5 g) were mixed with 10 mL of Lysis Buffer (50 mM MOPS $\text{pH } 7.5$, 2 mM dithio-

- 78 -

threitol (DTT), 2 mM benzamidine HCl, 5 μ M ZnSO₄, 0.1 mM CaCl₂, 20 μ g/mL calpain inhibitors I and II, and 5 μ g/mL each of leupeptin, pepstatin, and aprotinin) at room temperature. The cells were
5 lysed by passage through a French® pressure cell (SLM-Aminco®, Spectronic Instruments, Inc., Rochester NY). The resultant lysate was centrifuged in a Beckman ultracentrifuge using a type T180 rotor at 45,000 rpm for 1 hr. The supernatant was recovered
10 and filtered through a 0.2 μ m filter. This filtrate was applied to a 2.6 X 90 cm column of SEPHACRYL® S-300 equilibrated in Column Buffer A (Lysis Buffer containing 100 mM NaCl, and 2 mM MgCl₂). The column flow rate was adjusted to 1 mL/min and fractions of
15 7 mL were collected. Active fractions were pooled and supplemented with 0.16 mg of calmodulin. The enzyme was applied overnight at a flow rate of 0.2 mL/min to an ACC-1 agarose immunoaffinity column as described in Hansen et al., *Methods in Enzymology*
20 159, pp. 453-557 (1988). The column was washed with 5 volumes of Column Buffer B (Column Buffer A without NaCl) and followed by 5 volumes of Column Buffer C (Column Buffer A containing 250 mM NaCl). The column was eluted with Column Buffer D (50 mM MOPS
25 pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM benzamidine HCl, 100 mM NaCl, 20 μ g/mL calpain inhibitors I and II, and 5 μ g/mL each of leupeptin, pepstatin, and aprotinin) by applying one column volume at 0.1 mL/min, stopping flow for 1 hour, and
30 then continuing elution at the same flow rate. Fractions of 0.5 mL were collected. Fractions displaying activity were pooled, and first dialyzed against dialysis buffer containing 25 mM MOPS pH

- 79 -

7.5, 100 mM NaCl, 10 μ M ZnSO₄, 1 mM CaCl₂, 1 mM DTT, and 1 mM benzamidine HCl. A subsequent dialysis against dialysis buffer containing 50% glycerol was performed prior to quick-freezing the sample with dry ice and storage at -70°C. The resultant preparations were about 10 to 15% pure by SDS-PAGE. These preparations had specific activities of about 5 to 20 μ mol cAMP hydrolyzed per minute per milligram protein.

10

Purification of PDE1B from *S. cerevisiae*

Yeast cells (50 g) were thawed by mixing with 100 mL glass beads (0.5 mM, acid washed) and 200 mL Buffer A at room temperature. Buffer A consisted of 50 mM MOPS pH 7.5, 1 mM DTT, 2 mM benzamidine HCl, 0.01 mM ZnSO₄, 5 mM MgCl₂, 20 μ g/mL calpain inhibitors I and II, and 5 μ g/mL each of leupeptin, pepstatin, and aprotinin. The mixture was cooled to 4°C, transferred to a Bead-Beater®, and the cells lysed by rapid mixing for 6 cycles of 30 seconds each. The homogenate was centrifuged for 15 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C. The supernatant was recovered and PDE1B was precipitated by the addition of solid ammonium sulfate (0.33 g/mL supernatant) while stirring in an ice bath and maintaining the pH between 7.0 and 7.5. This mixture then was centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant

- 80 -

was discarded and the pellet was dissolved in 100 mL of buffer B (50 mM MOPS pH 7.5, 1 mM DTT, 1 mM benzamidine HCl, 0.01 mM ZnSO₄, 2 mM MgCl₂, 2 mM CaCl₂, and 5 µg/mL each of leupeptin, pepstatin, and aprotinin). The pH and conductivity were corrected to 7.5 and 15-20 milliSiemens (mS), respectively. This solution was loaded onto a 20 mL column of calmodulin-Agarose that had been equilibrated with 10 column volumes of Buffer B at a rate of 1 mL/min. The flow-through was reapplied to the column at least 5 times. The column was washed with 5 volumes of Buffer B, 5 volumes of buffer B containing 250 mM NaCl, and 2 volumes of Buffer B without NaCl again. Elution was accomplished by applying one volume of Buffer C (50 mM MOPS pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM benzamidine HCl) at 0.33 mL/min, then stopping flow for 1 hour before continuing the elution. Fractions of about 4 mL were collected and assayed for PDE activity. Active fractions were pooled and concentrated to a volume of 5 mL, using an Amicon ultrafiltration system. The concentrate was then applied to a 320 mL Sephacryl® S-300 column (1.6 X 150 cm) that had been equilibrated with at least 2 volumes of Buffer D (25 mM MOPS pH 7.5, 1 mM DTT, 1 mM benzamidine HCl, 0.01 mM ZnSO₄, 2 mM CaCl₂, and 100 mM NaCl). The column was developed at a flow rate of 1 mL/min (11 cm/hr), and 5 mL fractions were collected. The activity peak was pooled and dialyzed overnight against Buffer D containing 50% glycerol. The purified enzyme was frozen on dry ice and stored at -70°C. The resultant preparations were about >90% pure by SDS-PAGE. These prepara-

- 81 -

tions had specific activities of about 10 to 30 μmol cGMP hydrolyzed per minute per milligram protein.

Purification of PDE1C3 from Sf9 Cells

5

Cell pellets (5 g) were thawed on ice with 20 mL of Lysis Buffer (50 mM MOPS pH 7.4, 10 μM ZnSO_4 , 0.1 mM CaCl_2 , 1 mM DTT, 2 mM benzamidine HCl, 5 $\mu\text{g/mL}$ each of pepstatin, leupeptin, and aprotin-
10 in). Cells were lysed by passage through a French® pressure cell (SLM-Aminco®, Spectronic Instruments) while temperatures were maintained below 10°C. The resultant cell homogenate was centrifuged at 36,000 rpm at 4°C for 45 min in a Beckman ultracentrifuge
15 using a Type TI45 rotor. The supernatant was discarded and the resultant pellet was resuspended with 40 mL of Solubilization Buffer (Lysis Buffer containing 1 M NaCl, 0.1 M MgCl_2 , 1 mM CaCl_2 , 20 $\mu\text{g/mL}$ calmodulin, and 1% SulfoBetaine SB12 (Z3-12) by
20 sonicating using a VibraCell tuner with a microtip for 3 X 30 seconds. This was performed in a crushed ice/salt mix for cooling. Following sonication, the mixture was slowly mixed for 30 minutes at 4°C to finish solubilizing membrane bound proteins. This
25 mixture was centrifuged in a Beckman ultracentrifuge using a type TI45 rotor at 36,000 rpm for 45 minutes. The supernatant was diluted with Lysis Buffer containing 10 $\mu\text{g/mL}$ calpain inhibitors I and II. The precipitated protein was centrifuged for 20
30 minutes at 9,000 rpm in a Beckman JA-10 rotor. The recovered supernatant then was subjected to Mimetic Blue® AP Agarose Chromatography.

- 82 -

To run the Mimetic Blue® AP Agarose Column, the resin initially was shielded by the application of 10 bed volumes of 1% polyvinylpyrrolidone (i.e., MW of 40,000) to block nonspecific binding sites. The loosely bound PVP-40 was removed by washing with 10 bed volumes of 2 M NaCl, and 10 mM sodium citrate pH 3.4. Just prior to addition of the solubilized PCE1C3 sample, the column was equilibrated with 5 bed volumes of Column Buffer A (50 mM MOPS pH 7.4, 10 μ M ZnSO₄, 5 mM MgCl₂, 0.1 mM CaCl₂, 1 mM DTT, 2 mM benzamidine HCl).

The solubilized sample was applied to the column at a flow rate of 2 mL/min with recycling such that the total sample was applied 4 to 5 times in 12 hours. After loading was completed, the column was washed with 10 column volumes of Column Buffer A, followed by 5 column volumes of Column Buffer B (Column Buffer A containing 20 mM 5'-AMP), and followed by 5 column volumes of Column Buffer C (50 mM MOPS pH 7.4, 10 μ M ZnSO₄, 0.1 mM CaCl₂, 1 mM DTT, and 2 mM benzamidine HCl). The enzyme was eluted into three successive pools. The first pool consisted of enzyme from a 5-bed volume wash with Column Buffer C containing 1 mM cAMP. The second pool consisted of enzyme from a 10-bed volume wash with Column Buffer C containing 1 M NaCl. The final pool of enzyme consisted of a 5-bed volume wash with Column Buffer C containing 1 M NaCl and 20 mM cAMP.

The active pools of enzyme were collected and the cyclic nucleotide removed via conventional gel filtration chromatography or chromatography on hydroxyapatite resins. Following removal of cyclic nucleotides, the enzyme pools were dialyzed against

- 83 -

Dialysis Buffer containing 25 mM MOPS pH 7.4, 10 μ M ZnSO₄, 500 mM NaCl, 1 mM CaCl₂, 1 mM DTT, 1 mM benzamidine HCl, followed by dialysis against Dialysis buffer containing 50% glycerol. The enzyme was quick-frozen with the aid of dry ice and stored at -70°C.

The resultant preparations were about >90% pure by SDS-PAGE. These preparations had specific activities of about 0.1 to 1.0 μ mol cAMP hydrolyzed per minute per milligram protein.

Purification of PDE2 from *S. cerevisiae*

Frozen yeast cell pellets from strain YI34 (10g, stored at -70°C) were allowed to thaw on ice in 25 mL of Lysis Buffer (50 mM MOPS, pH 7.2, 1 mM EDTA, 1 mM EGTA, 0.1 mM DTT, 0.1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), 1 μ g/mL of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II, and 2 mM benzamidine). Cells were lysed by three passages through a French® pressure cell (SLM-Aminco®, Spectronic Instruments). The lysate was centrifuged at 36,000 rpm in a Beckman Ultracentrifuge rotor Type 45Ti for 60 minutes at 4°C. The supernatant was separated from sediment and passed through a 15 mL Epoxy-cGMP Sepharos® resin at 4°C two times at about 0.5 mL/min. The column subsequently was washed with 45 mL of Wash Buffer 1 (50 mM MOPS, pH 7.2, 0.1 mM EDTA, 0.1 mM DTT). Following this wash, the column was washed with 45 mL of Wash Buffer 2 (Wash Buffer 1 containing 0.5 M NaCl). Following this salt wash, the column was washed with 15 mL of Wash Buffer 3 (Wash Buffer 1 containing

- 84 -

0.25 M NaCl). The column was transferred to room temperature and allowed to warm. Approximately 25 mL of Elution Buffer (Wash Buffer 3 containing 10 mM cGMP, maintained at room temperature) was applied to the column and the effluent was collected in 2 mL fractions. Small aliquots of each of the fractions were diluted 20-fold in PBS containing 5 mM MgCl₂ to allow hydrolysis of the competing ligand and to aid detection of PDE2 activity. Active fractions were passed through a Pharmacia PD-10[®] gel filtration column to exchange into Wash Buffer 3. This exchanged pool was diluted 50% v/v with sterile 80% glycerol and stored at -20°C. The resultant preparations were greater than 85% pure as judged by SDS-PAGE with subsequent staining of protein by Coomassie R-250. These preparations had specific activities of about 150 to 250 μ mol cGMP hydrolyzed per minute per milligram protein.

20 Preparation of PDE3A from Sf9 Cells

Cells (2 X 10¹⁰) were suspended in Lysis Buffer containing 50 mM MOPS pH 7.5, 2 mM DTT, 2 mM benzamidine HCl, 5 μ M ZnSO₄, 0.1 mM CaCl₂, 20 μ g/mL calpain inhibitors I and II, and 5 μ g/mL each of leupeptin, pepstatin, and aprotinin. The mixture was sonicated twice for 30 seconds and the cells were lysed in a French[®] pressure cell (SLM-Aminco[®], Spectronic Instruments) at 4°C. The lysate was centrifuged 100,000 X g for 45 minutes. The pellet was washed once in Lysis Buffer and suspended in 46 mL Lysis Buffer with a Dounce homogenizer. Aliquots were stored at -70°C. These preparations had spe-

- 85 -

cific activities of about 1 to 2 nmol cAMP hydrolyzed per minute per milligram protein.

Human PDE4A, 4B, 4C, 4D Preparations

5

Preparation of PDE4A from *S. cerevisiae*

Yeast cells (50 g of yeast strain YI26 harboring HDUN1.46) were thawed at room temperature
10 by mixing with 50 mL of Lysis Buffer (50 mM MOPS pH 7.5, 10 μ M ZnSO₄, 2 mM MgCl₂, 14.2 mM 2-mercaptoethanol, 5 μ g/mL each of pepstatin, leupeptin, aprotinin, 20 μ g/mL each of calpain inhibitors I and II, and 2 mM benzamidine HCl). Cells were lysed in
15 a French® pressure cell (SLM-Aminco®, Spectronic Instruments) at 10°C. The extract was centrifuged in a Beckman JA-10 rotor at 9,000 rpm for 22 minutes at 4°C. The supernatant was removed and centrifuged in a Beckman TI45 rotor at 36,000 rpm for 45 minutes
20 at 4°C.

PDE4A was precipitated from the high-speed supernatant by the addition of solid ammonium sulfate (0.26 g/mL supernatant) while stirring in an ice bath and maintaining the pH between 7.0 and 7.5.
25 The precipitated proteins containing PDE4A were collected via centrifugation in a Beckman JA-10 rotor at 9,000 rpm for 22 minutes. The precipitate was resuspended in 50 mL of Buffer G (50 mM MOPS pH 7.5, 10 μ M ZnSO₄, 5 mM MgCl₂, 100 mM NaCl, 14.2 mM 2-
30 mercaptoethanol, 2 mM benzamidine HCl, 5 μ g/mL each of leupeptin, pepstatin, and aprotinin, and 20 μ g/mL each of calpain inhibitors I and II) and passed through a 0.45 μ m filter.

- 86 -

The resuspended sample (50 to 100 mL) was loaded onto a 5 X 100 cm column of Pharmacia SEPHACRYL® S-300 equilibrated in Buffer G. Enzyme activity was eluted at a flow rate of 2 mL/min and
5 pooled for later fractionation.

The PDE4A isolated from gel filtration chromatography was applied to a 1.6 X 20 cm column of Sigma Cibacron Blue Agarose-type 300 (10 mL) equilibrated in Buffer A (50 mM MOPS pH 7.5, 10 μ M
10 ZnSO₄, 5 mM MgCl₂, 14.2 mM 2-mercaptoethanol, and 100 mM benzamidine HCl). The column was washed in succession with 50 to 100 mL of Buffer A, 20 to 30 mL of Buffer A containing 20 mM 5'-AMP, 50 to 100 mL of Buffer A containing 1.5 M NaCl, and 10 to 20 mL of
15 Buffer C (50 mM Tris HCl pH 8, 10 μ M ZnSO₄, 14.2 mM 2-mercaptoethanol, and 2 mM benzamidine HCl). The enzyme was eluted with 20 to 30 mL of Buffer C containing 20 mM cAMP.

The PDE activity peak was pooled, and
20 precipitated with ammonium sulfate (0.33 g/mL enzyme pool) to remove excess cyclic nucleotide. The precipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.5, 5 μ M ZnSO₄, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidine HCl), and desalted via gel
25 filtration on a Pharmacia PD-10® column per manufacturer's instructions. The enzyme was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >80% pure by SDS-PAGE. These preparations had specific
30 activities of about 10 to 40 μ mol cAMP hydrolyzed per minute per milligram protein.

- 87 -

Preparation of PDE4B from *S. cerevisiae*

Yeast cells (150 g of yeast strain YI23 harboring HDUN2.32) were thawed by mixing with 100 mL glass beads (0.5 mM, acid washed) and 150 mL Lysis Buffer (50 mM MOPS pH 7.2, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, 5 μ g/mL each of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II) at room temperature. The mixture was cooled to 4°C, transferred to a Bead-Beater®, and the cells lysed by rapid mixing for 6 cycles of 30 seconds each. The homogenate was centrifuged for 22 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C. The supernatant was recovered and PDE4B was precipitated by the addition of solid ammonium sulfate (0.26 g/mL supernatant) while stirring in an ice bath and maintaining the pH between 7.0 and 7.5. This mixture was then centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant was discarded and the pellet was dissolved in 200 mL of Buffer A (50 mM MOPS pH 7.5, 5 mM MgCl₂, 1 mM DTT, 1 mM benzamidine HCl, and 5 μ g/mL each of leupeptin, pepstatin, and aprotinin). The pH and conductivity were corrected to 7.5 and 15-20 mS, respectively.

The resuspended sample was loaded onto a 1.6 X 200 cm column (25 mL) of Sigma Cibacron Blue Agarose-type 300 equilibrated in Buffer A. The sample was cycled through the column 4 to 6 times over the course of 12 hours. The column was washed

- 88 -

in succession with 125 to 250 mL of Buffer A, 125 to 250 mL of Buffer A containing 1.5 M NaCl, and 25 to 50 mL of Buffer A. The enzyme was eluted with 50 to 75 mL of Buffer E (50 mM Tris HCl pH 8, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, and 20 mM cAMP) and 50 to 75 mL of Buffer E containing 1 M NaCl. The PDE activity peak was pooled, and precipitated with ammonium sulfate (0.4 g/mL enzyme pool) to remove excess cyclic nucleotide. The precipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.5, 5 μ M ZnSO₄, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidine HCl) and desalted via gel filtration on a Pharmacia PD-10[®] column per manufacturer's instructions. The enzyme pool was dialyzed overnight against Buffer X containing 50% glycerol. This enzyme was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >90% pure by SDS-PAGE. These preparations had specific activities of about 10 to 50 μ mol cAMP hydrolyzed per minute per milligram protein.

Preparation of PDE4C from *S. cerevisiae*

Yeast cells (150 g of yeast strain Y130 harboring HDUN3.48) were thawed by mixing with 100 mL glass beads (0.5 mM, acid washed) and 150 mL Lysis Buffer (50 mM MOPS pH 7.2, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, 5 μ g/mL each of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II) at room temperature. The mixture was cooled to 4°C, transferred to a BEAD-BEATER[®], and the cells lysed by rapid mixing for 6 cycles of 30

- 89 -

sec each. The homogenate was centrifuged for 22 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultra-
5 centrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C.

The supernatant was recovered and PDE4C was precipitated by the addition of solid ammonium sulfate (0.26 g/mL supernatant) while stirring in an
10 ice bath and maintaining the pH between 7.0 and 7.5. Thirty minutes later, this mixture was centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant was discarded and the pellet was dissolved in
15 200 mL of Buffer A (50 mM MOPS pH 7.5, 5 mM MgCl₂, 1 mM DTT, 2 mM benzamidine HCl, and 5 µg/mL each of leupeptin, pepstatin, and aprotinin). The pH and conductivity were corrected to 7.5 and 15-20 mS, respectively.

20 The resuspended sample was loaded onto a 1.6 X 20 cm column (25 mL) of Sigma Cibacron Blue Agarose-type 300 equilibrated in Buffer A. The sample was cycled through the column 4 to 6 times over the course of 12 hours. The column was washed
25 in succession with 125 to 250 mL of Buffer A, 125 to 250 mL of Buffer A containing 1.5 M NaCl, and then 25 to 50 mL of Buffer A. The enzyme was eluted with 50 to 75 mL of Buffer E (50 mM Tris HCl pH 8, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, and
30 20 mM cAMP) and 50 to 75 mL of Buffer E containing 1 M NaCl. The PDE4C activity peak was pooled, and precipitated with ammonium sulfate (0.4 g/mL enzyme pool) to remove excess cyclic nucleotide. The pre-

- 90 -

cipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.2, 5 μ M ZnSO₄, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidine HCl) and desalted via gel filtration on a Pharmacia PD-10[®] column per manufacturer's instructions. The enzyme pool was dialyzed overnight against Buffer X containing 50% glycerol. This enzyme was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >80% pure by SDS-PAGE. These preparations had specific activities of about 10 to 20 μ mol cAMP hydrolyzed per minute per milligram protein.

Preparation of PDE4D from *S. cerevisiae*

Yeast cells (100 g of yeast strain YI29 harboring HDUN4.11) were thawed by mixing with 150 mL glass beads (0.5 mM, acid washed) and 150 mL Lysis Buffer (50 mM MOPS pH 7.2, 10 μ M ZnSO₄, 2 mM MgCl₂, 14.2 mM 2-mercaptoethanol, 2 mM benzamidine HCl, 5 μ g/mL each of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II) at room temperature. The mixture was cooled to 4°C, transferred to a Bead-Beater[®], and the cells lysed by rapid mixing for 6 cycles of 30 sec each. The homogenate was centrifuged for 22 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C. The supernatant was recovered and PDE4D was precipitated by the addition of solid ammonium sulfate (0.33 g/mL supernatant) while stirring in an ice bath and maintain-

- 91 -

ing the pH between 7.0 and 7.5. Thirty minutes later, this mixture was centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant was discarded and the pellet was dissolved in 100 mL of Buffer A (50 mM MOPS pH 7.5, 10 μ M ZnSO₄, 5 mM MgCl₂, 14.2 mM 2-mercaptoethanol, 100 mM benzamidinium HCl, and 5 μ g/mL each of leupeptin, pepstatin, aprotinin, calpain inhibitor I and II). The pH and conductivity were corrected to 7.5 and 15-20 mS, respectively.

At a flow rate of 0.67 mL/min, the resuspended sample was loaded onto a 1.6 X 20 cm column (10 mL) of Sigma Cibacron Blue Agarose-type 300 equilibrated in Buffer A. The column was washed in succession with 50 to 100 mL of Buffer A, 20 to 30 mL of Buffer A containing 20 mM 5'-AMP, 50 to 100 mL of Buffer A containing 1.5 M NaCl, and then 10 to 20 mL of Buffer C (50 mM Tris HCl pH 8, 10 μ M ZnSO₄, 14.2 mM 2-mercaptoethanol, 2 mM benzamidinium HCl). The enzyme was eluted with 20 to 30 mL of Buffer C containing 20 mM cAMP.

The PDE4D activity peak was pooled and precipitated with ammonium sulfate (0.4 g/mL enzyme pool) to remove excess cyclic nucleotide. The precipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.2, 5 μ M ZnSO₄, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidinium HCl) and desalted via gel filtration on a Pharmacia PD-10[®] column per manufacturer's instructions. The enzyme pool was dialyzed overnight against Buffer X containing 50% glycerol. This enzyme preparation was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

- 92 -

The resultant preparations were about >80% pure by SDS-PAGE. These preparations had specific activities of about 20 to 50 μmol cAMP hydrolyzed per minute per milligram protein.

5

Purification of PDE5 from *S. cerevisiae*

Cell pellets (29 g) were thawed on ice with an equal volume of Lysis Buffer (25 mM Tris HCl, pH 8, 5 mM MgCl_2 , 0.25 mM DTT, 1 mM benzamidine, and 10 μM ZnSO_4). Cells were lysed in a Microfluidizer® (Microfluidics Corp.) using nitrogen at 20,000 psi. The lysate was centrifuged and filtered through 0.45 μm disposable filters. The filtrate was applied to a 150 mL column of Q SEPHAROSE® Fast-Flow (Pharmacia). The column was washed with 1.5 volumes of Buffer A (20 mM Bis-Tris Propane, pH 6.8, 1 mM MgCl_2 , 0.25 mM DTT, 10 μM ZnSO_4) and eluted with a step gradient of 125 mM NaCl in Buffer A followed by a linear gradient of 125-1000 mM NaCl in Buffer A. Active fractions from the linear gradient were applied to a 180 mL hydroxyapatite column in Buffer B (20 mM Bis-Tris Propane (pH 6.8), 1 mM MgCl_2 , 0.25 mM DTT, 10 μM ZnSO_4 , and 250 mM KCl). After loading, the column was washed with 2 volumes of Buffer B and eluted with a linear gradient of 0-125 mM potassium phosphate in Buffer B. Active fractions were pooled, precipitated with 60% ammonium sulfate, and resuspended in Buffer C (20 mM Bis-Tris Propane, pH 6.8, 125 mM NaCl, 0.5 mM DTT, and 10 μM ZnSO_4). The pool was applied to a 140 mL column of SEPHACRYL® S-300 HR and eluted with Buffer C. Ac-

- 93 -

tive fractions were diluted to 50% glycerol and stored at -20°C.

The resultant preparations were about 85% pure by SDS-PAGE. These preparations had specific activities of about 3 μ mol cGMP hydrolyzed per minute per milligram protein.

Preparation of PDE7 from *S. cerevisiae*

10 Cell pellets (126 g) were thawed and re-suspended at room temperature for about 30 minutes with an equal volume of Lysis Buffer (50 mM Tris HCl, pH 8, 1 mM EDTA, 1 mM DTT, 50 mM NaCl, 2 mM benzamidine HCl, and 5 μ g/mL each of pepstatin, 15 leupeptin, and aprotinin). The cells were lysed at 0-4°C with the aid of glass beads (125 mL) in a Bead-Beater® for 6 X 30 second cycles. The lysate was centrifuged and filtered through 0.45 μ m disposable filters. The filtered extract (178 mL) was 20 distributed into 4 mL aliquots, quick-frozen with dry ice, and stored in a freezer at -70°C. These preparations were stable to several cycles of freezing and thawing and had specific activities of about 50 to 100 pmol cAMP hydrolyzed per minute per milli- 25 gram protein.

Lipopolysaccharide-Stimulated TNF α Release from Human Peripheral Blood Lymphocytes

30 To assess the ability of a compound to reduce TNF α secretion in human peripheral blood lymphocytes (PBL), the following tests were performed. Previous studies have demonstrated that

- 94 -

incubation of human PBL with cAMP-elevating agents, such as prostaglandin E21 forskolin, 8-bromo-cAMP, or dibutryl-cAMP, inhibits the secretion of TNF α by the cells when stimulated by lipopolysaccharide (LPS; endotoxin). Accordingly, preliminary experiments have been performed to demonstrate that selective PDE4 inhibitors, such as rolipram, inhibit LPS-induced TNF α secretion from human lymphocytes in a dose-dependent fashion. Hence, TNF α secretion from human PBL was used as a standard for the ability of a compound to elevate intracellular cAMP concentrations and/or to inhibit PDE4 activity within the cell.

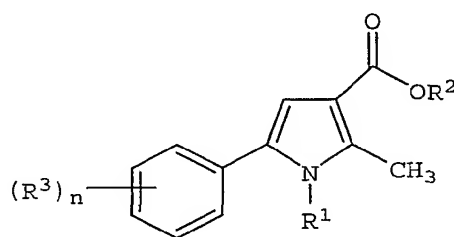
Heparinized blood (approximately 30 mL) drawn from human volunteers was mixed 1:1 with Dulbecco's modified phosphate-buffered saline. This mixture was mixed 1:1 with HISTOPAQUE[®] and centrifuged at 1,500 rpm at room temperature without braking in the swinging bucket of a Beckman model TJ6 centrifuge. Erythrocytes were centrifuged to the bottom of the tubes, and serum remained at the surface of the tubes. A layer containing lymphocytes sedimented between the serum and HISTOPAQUE[®] layers, and was removed by aspiration to a fresh tube. The cells were quantified and adjusted to 3×10^6 cells/mL and a 100 μ L aliquot is placed into the wells of a 96 well plate. Test compounds and RPMI media (Gibco/BRL Life Sciences) are added to each of the wells 15 minutes prior to addition of bacterial LPS (25 mg/mL). The mixture was allowed to incubate for 20 hours at 37°C in a humidified chamber. The cells then were separated by centrifuging at 800 rpm for 5 minutes at room temperature. An aliquot of

- 95 -

180 μ L of supernatant was transferred to a new plate for determination of TNF α concentration. TNF α protein in the cell supernatant fluids was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (CYTOSCREEN[®] Immunoassay Kit from Biosource International).

The cell-based assay provided the following results for various compounds of the present invention. The EC₅₀ values (i.e., effective concentration of the compound capable of inhibiting 50% of the total TNF α) illustrate the ability of the present compounds to inhibit LPS-stimulated TNF α release from human PBL.

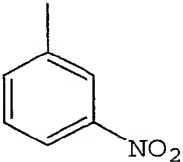
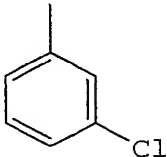
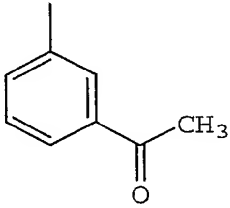
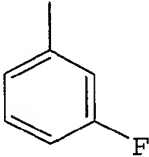
The following summarizes the IC₅₀ values determined for compounds of structural formulae (I) and (II) against human recombinant PDE4. In the following table, R³ is hydrogen and n is 1, unless otherwise noted.

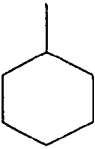
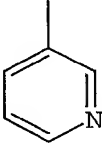
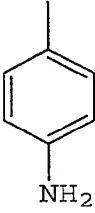
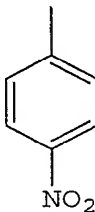


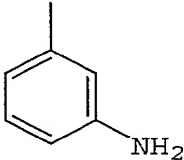
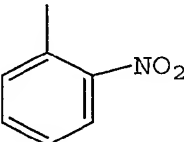
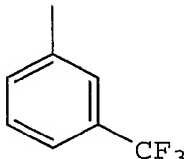
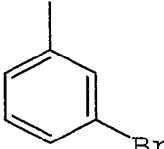
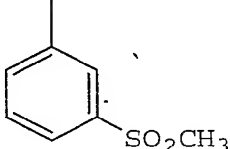
(I)

Et=ethyl

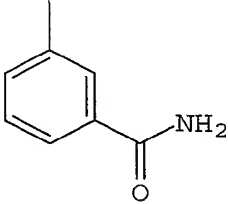
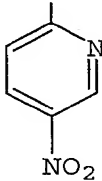
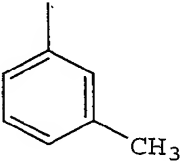
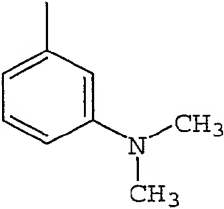
H=hydrogen

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
22	 C ₆ H ₄ NO ₂	Et	0.15
3	 C ₆ H ₄ Cl	Et	0.20
5 8	 C ₈ H ₇ O	Et	0.59
18	 C ₆ H ₄ F	Et	0.62

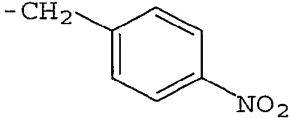
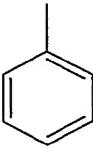
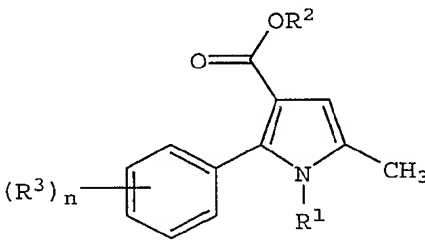
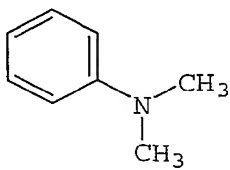
Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
20	 C ₆ H ₁₁	Et	0.99
19	 C ₃ H ₄ N	Et	1.28
38	 C ₆ H ₆ N	Et	1.30
33	 C ₆ H ₄ NO ₂	Et	1.40

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
41	 C ₆ H ₆ N	Et	1.42
34	 C ₆ H ₄ NO ₂	Et	1.65
28	 C ₇ H ₄ F ₃	Et	2.28
26	 C ₆ H ₄ Br	Et	3.07
10	 C ₇ H ₇ O ₂ S	Et	4.38

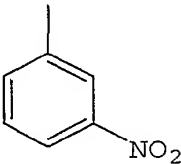
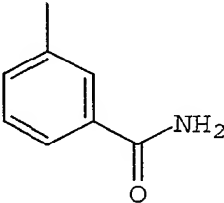
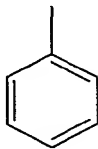
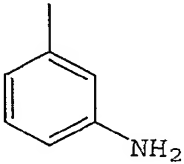
- 99 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
11	 C ₇ H ₆ NO	Et	5.45
35	 C ₅ H ₃ N ₂ O ₂	Et	5.74
5	 C ₇ H ₇	Et	6.10
1	 C ₈ H ₁₀ N	Et	8.22

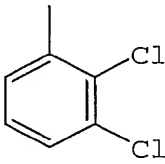
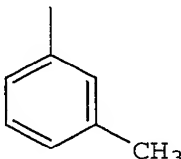
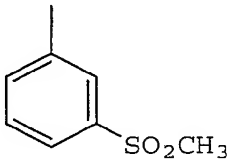
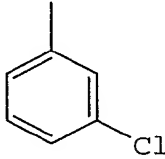
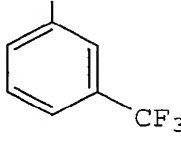
- 100 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
30	 $C_7H_5N_2O_4$	Et	13.42
44	 C_6H_5	H	54.16
5	 (II) Et=ethyl H=hydrogen		
10			
15			
20			
13	 $C_8H_{10}N$	Et	0.15

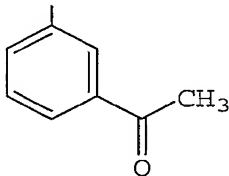
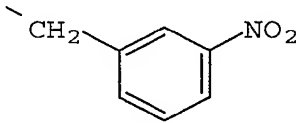
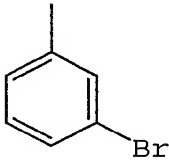
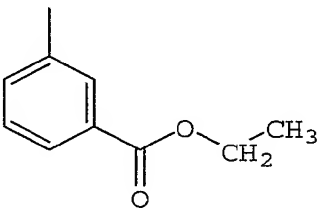
- 101 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
39	 C ₆ H ₄ NO ₂	Et	0.20
12	 C ₇ H ₆ NO	Et	0.22
21	 C ₆ H ₅	Et	0.59
40	 C ₆ H ₅ N	Et	1.29

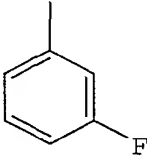
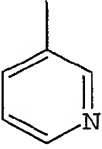
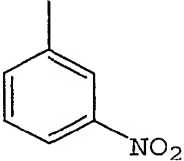
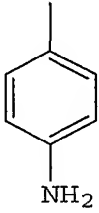
- 102 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
42	 <chem>Cc1cc(Cl)c(Cl)cc1</chem> $C_6H_3Cl_2$	Et	1.49
4	 <chem>Cc1cccc(C)c1</chem> C_7H_8	Et	2.90
9	 <chem>Cc1cccc(S(=O)(=O)C)c1</chem> $C_7H_7O_2S$	Et	4.58
2	 <chem>Cc1cccc(Cl)c1</chem> C_6H_4Cl	Et	5.45
29	 <chem>Cc1cccc(C(F)(F)F)c1</chem> $C_7H_4F_3$	Et	6.78

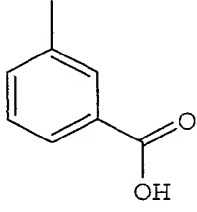
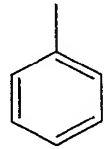
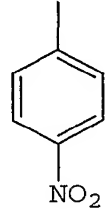
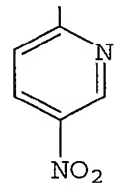
- 103 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
7	 C ₈ H ₇ O	Et	6.83
31	 C ₇ H ₆ NO ₂	Et	5.13
6	 C ₆ H ₄ Br	Et	8.55
46	 C ₉ H ₉ O ₂	Et	8.70

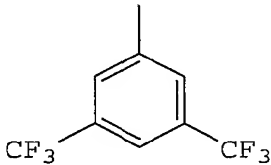
- 104 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
17	 C ₆ H ₄ F	Et	13.21
32	 C ₅ H ₄ N	Et	13.39
43	 C ₆ H ₄ NO ₂	H	15.63
37	 C ₆ H ₅ N	Et	16.00

- 105 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
24	 C ₇ H ₅ O ₂	Et	16.53
21	 C ₆ H ₅	Et	31.29
36	 C ₆ H ₄ NO ₂	Et	110.00
23	 C ₅ H ₃ N ₂ O ₂	Et	133.01

- 106 -

Example	R ¹	R ²	PDE4 IC ₅₀ (μ M)
45	 C ₈ H ₇ F ₆	Et	193.71

5 The data presented above shows that compounds of formula (I) and (II) are potent and selective inhibitors of PDE4, e.g., the compounds have an IC₅₀ vs. human recombinant PDE4 of about 0.15 to about 195 μ m. As an added advantage, the compounds

10 of formula (I) and (II) reduced or eliminated the adverse side effects, such as CNS effects, associated with prior PDE4 inhibitors. In particular, compounds of formulae (I) and (II) were tested in cell-based assays and in animal models to illustrate

15 the efficacy of the compounds with respect to inhibiting PDE4 both *in vitro* and *in vivo*.

 The cell-based assay provided the following results for various pyrrole compounds of the present invention. The EC₅₀ values (i.e., effective

20 concentration of the compound capable of inhibiting 50% of the total TNF α) illustrate the ability of the present compounds to inhibit LPS-stimulated TNF α release from human peripheral blood lymphocytes.

- 107 -

Compound	PDE4 IC ₅₀ (μm)	PBL/TNFα EC ₅₀ (μM)
Ex. 22	.15	.87
Ex. 3	.20	1.50
Ex. 21	.40	10.00
Ex. 40	1.29	6.72
Ex. 42	1.49	100.0

10 The above Table and FIG. 1 illustrate the ability of compounds of formulae (I) and (II) to inhibit PDE4 activity and TNFα release. Preferred compounds have a PBL/TNFα EC₅₀ about 500 nM or less, and preferably about 200 nM or less. More preferred compounds have
15 a PBL/TNFα EC₅₀ of about 100 nM or less.

To achieve the full advantages of the present invention, the compound has an IC₅₀ vs. human recombinant PDE4 of about 100 nM or less and a PBL/TNFα EC₅₀ of about 500 nM or less. More preferably,
20 bly, the compound has an IC₅₀ of about 50 nM or less and a PBL/TNFα EC₅₀ of about 100 nM or less.

- 108 -

Animal ModelsAssay for Inhibition of
Serum TNF α Levels in Mammals
5 (Mouse/TNF α ED₅₀ (mg/kg))

In order to assess the ability of a compound to reduce serum TNF α levels in mammals, the following protocol was employed. Those skilled in the art appreciate that previous studies have demonstrated that incubation of LPS-activated human monocytes with agents that can elevate cAMP, like PGE₂, forskolin, and the dbcAMP, inhibited secretion of TNF α . PDE4 inhibitors like rolipram, which also elevate cAMP, have been found to inhibit serum TNF α as well. Rolipram has also been found to inhibit secretion of TNF α from LPS-activated mouse macrophages. Accordingly, *in vivo* efficacy of a PDE4 reducing compound was shown by dosing with compound and measuring reduction of serum TNF α levels in LPS-injected mice. Female C3H mice, 20-25 gm body weight, were fasted overnight and dosed intraperitoneally with test compound in appropriate vehicle 60 minutes before LPS injection. Five μ g of LPS was then injected intraperitoneally into the mice. Ninety minutes after LPS injection, mice were bled from the heart. Blood was allowed to clot overnight at 4°C. Samples were centrifuged for 10 minutes in a microcentrifuge and the serum removed and stored at -20°C until analysis. Serum levels of TNF α were subsequently measured using a commercially available ELISA kit (Genzyme) following the protocol enclosed in the kit. The inhibition of serum TNF α levels

- 109 -

caused by the compound was determined relative to serum TNF α levels in control mice receiving vehicle alone. The results are summarized in the plots of FIG. 1.

5

Combined Mouse endotoxin-stimulated TNF α
Release and Locomotor Activity Assay

10 The purpose of this study was to determine the efficacy of PDE4 inhibitors *in vivo* in an LPS mouse model together with a determination with respect to central nervous system (CNS) side-effects manifested by a decrease in spontaneous mobility.

15 The test animals were female Balb/c mice, having an average weight of about 20 g. The PDE4 inhibitors, formulated in 30% Cremophor® EL, were administered via intraperitoneal (i.p.) injections at doses of 0.1, 1.0, 10.0, and 100 mg/kg. Individual dose volumes (about 150 μ L) were adjusted based
20 on the body weights measured. One hour later, 5 mg/kg LPS in a final volume of 200 μ L was injected via the tail vein to each animal. Ninety minutes following the LPS treatment, the animals were bled
25 and serum samples were collected before being stored at -70°C until assayed.

 For efficacy determination, the serum samples were diluted two-fold and TNF α levels were determined using the CYTOSCREEN® Immunoassay Kit
30 (Biosource International). The data were averaged between triplicate sample subjects for each of the tested compounds.

- 110 -

For side-effect profiling, a subjective visual scoring system was utilized at 5min and 20min after administration of PDE4 inhibitors. Vehicle control animals were rated a single "+" and animals that were effectively immobilized and stretched out on the bottom of the cage with little detectable movement were rated as "++++." Alternatively, a semi-automated "open field" system (e.g., a Photo-beam Activity Measurement System as sold by San Diego Instruments) for monitoring movements was used for assessing the effect of PDE4 inhibitors on mice and/or rats. In this instance, the subjects could be monitored continuously over pre-set intervals.

Movement of the X-Y plane, or rearing up on the hind legs, was quantified by counting the number of "light-beam" crosses per unit of time. A decrease in the number of activity events is directly proportional to the mobility or immobilization of the animal. The quantitative scoring correlated well with the subjective measurements described above.

Compound	Vehicle control	1 mg/kg	10 mg/kg	100 mg/kg
Comparative Example ¹⁾	3	3	3	3
Example 22		3	3	3

¹⁾ trans-3-(3-cyclopentoxy-4-methoxyphenyl)-1-methoxycarbonyl-4-methyl-4-(methylcarbonyl)pyrrolidine, i.e., Example 12 of Feldman et al. U.S. Patent No. 5,665,754, incorporated herein by reference.

- 111 -

	Compound No.	Doses (mg/kg)	Sedative Effect	%TNF α Inhibition
5	Comparative Example ¹⁾	1	--	43
		10	+	100
		100	+++	100
	Example 22	1	--	13
		10	--	84
		100	--	100

10 The data presented above show that compounds of formulae (I) and (II) are potent and selective inhibitors of PDE4. As an important added advantage, the compounds of formulae (I) and (II) also reduced or eliminated the adverse CNS side
15 effects associated with prior PDE4 inhibitors. Compounds of formulae (I) and (II) were further tested for emetogenic properties in animal models to further illustrate the efficacy of the compounds. The method and results of the emetogenic test are
20 set forth below.

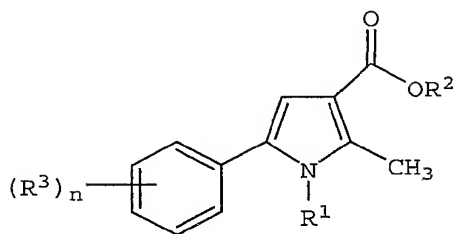
 The compounds of the present invention are useful for selectively inhibiting PDE4 activity in a mammal, without exhibiting the adverse CNS and emetic effects associated with prior PDE4 inhibitors.
25 tors.

 Obviously, many modifications and variations of the invention as hereinbefore set forth can be made without departing from the spirit and scope thereof and, therefore, only such limitations should
30 be imposed as are indicated by the appended claims.

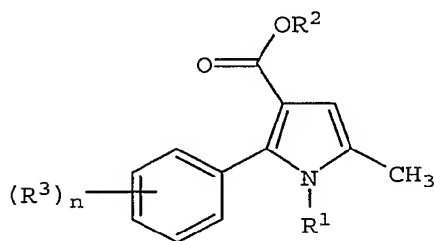
- 112 -

WHAT IS CLAIMED IS:

1. A compound having a formula



or



wherein R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)alkyl$, NR^4R^5 , $C(=O)NR^5R^6$, $C(=O)Oalkyl$, CO_2H , $OC(=O)alkyl$, nitro, halo, alkylthio, $SO_2(alkyl)$, SO_3H , and haloalkyl;

- 113 -

R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3,

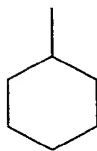
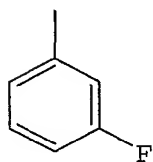
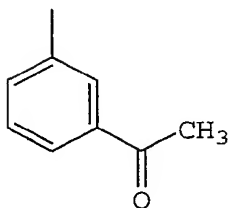
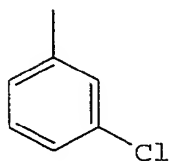
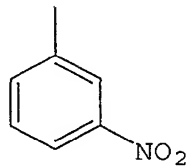
with the proviso that when R^2 is ethyl, R^1 is different from 3-nitrophenyl, 2,3-dichlorophenyl, and 3,5-bis-trifluoromethylphenyl.

2. The compound of claim 1 wherein R^1 is selected from the group consisting of aryl, heteroaryl, heteroalkaryl, alkaryl, and cycloalkyl.

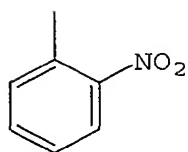
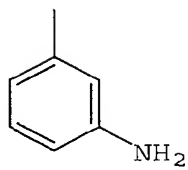
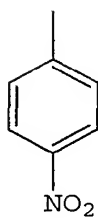
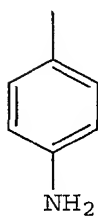
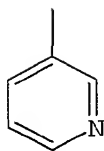
3. The composition of claim 1 wherein R^1 is selected from the group consisting of aryl, heteroaryl, cycloalkyl, and alkaryl, optionally substituted with one or more of NR^4R^5 , halo, alkyl, $C(=O)alkyl$, $SO_2(alkyl)$, $C(=O)NR^5R^6$, SO_3H , NO_2 , CO_2H , $C(=O)Oalkyl$, and CF_3 .

- 114 -

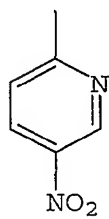
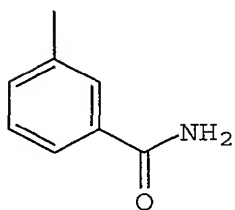
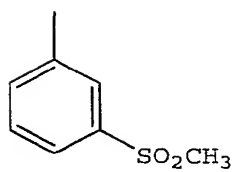
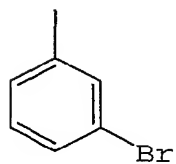
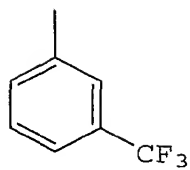
4. The compound of claim 1 wherein R¹ is selected from the group consisting of:



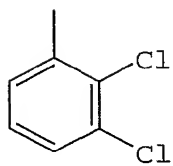
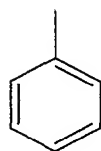
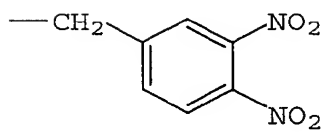
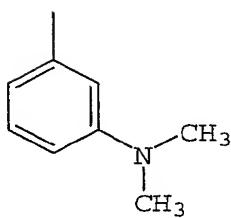
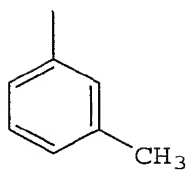
- 115 -



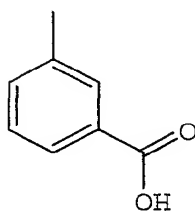
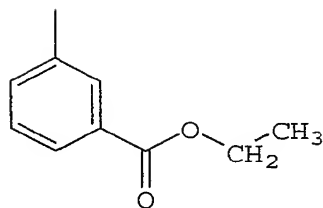
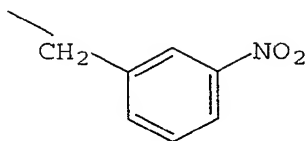
- 116 -



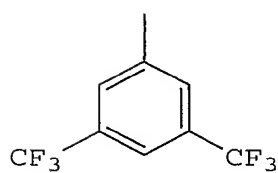
- 117 -



- 118 -



and



5. The composition of claim 1 wherein R² is selected from the group consisting of hydrogen and lower alkyl.

- 119 -

6. The composition of claim 1 wherein R¹ is phenyl, pyridyl, or cyclohexyl, optionally substituted; R² is hydrogen or C₁₋₂alkyl; and n is 0.

- 120 -

7. A compound of claim 1 selected from the group consisting of:

1-(3-dimethylaminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-chlorophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-2-phenyl-1-m-tolyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-5-phenyl-1-m-tolyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-bromophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-acetylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-acetylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-methanesulfonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-methanesulfonylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-dimethylaminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-iodophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-5-phenyl-1-(3-sulfophenyl)-1H-pyrrole-3-carboxylic acid ethyl ester;

- 121 -

5-methyl-2-phenyl-1-(3-sulfophenyl)-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-fluorophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-fluorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-5-phenyl-1-pyridin-3-yl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-cyclohexyl-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1-(5-nitropyridin-2-yl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-carboxyphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-methoxycarbonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-bromophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-iodophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-5-phenyl-1-(3-trifluoromethylphenyl)-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-2-phenyl-1-(3-trifluoromethylphenyl)-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(3-nitrobenzyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1-(3-nitrobenzyl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

- 122 -

5-methyl-2-phenyl-1-pyridin-3-yl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(4-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(2-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(5-nitropyridin-2-yl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1-(4-nitrophenyl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(4-aminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(4-aminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-aminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-aminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1-(3-nitrophenyl)-2-phenyl-1H-pyrrole-3-carboxylic acid;

5-methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid;
and

1-(3-ethoxycarbonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester.

- 123 -

8. The compound of claim 1 selected from the group consisting of:

1-(3-chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-acetylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-dimethylaminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-fluorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-cyclohexyl-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid ethyl ester; and

2-methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester.

- 124 -

9. The compound of claim 1 selected from the group consisting of:

1-(3-chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-dimethylaminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester; and

2-methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester.

10. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 0.5 μM to about 25 μM .

11. The compound of claim 1 having a PBL/TNF α EC_{50} of about 1 μM to about 20 μM .

12. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 0.5 μM to about 25 μM , and a PBL/TNF α EC_{50} of about 1 μM to about 20 μM .

13. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 100 μM or less.

14. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 50 μM or less.

15. The compound of claim 1 having a PBL/TNF α EC_{50} of about 500 μM or less.

- 125 -

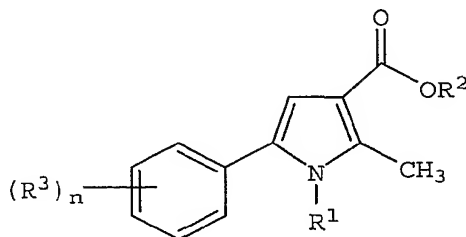
16. The compound of claim 1 having a PBL/TNF α EC₅₀ of about 100 μ M or less.

17. The compound of claim 1 having an IC₅₀ vs. human recombinant PDE4 of about 100 μ M or less and a PBL/TNF α EC₅₀ of about 500 μ M or less.

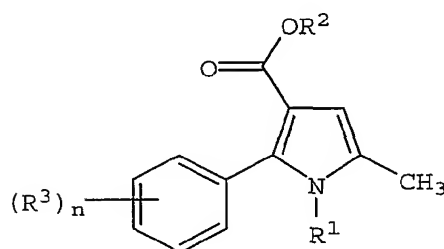
18. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

- 126 -

19. A method of treating a mammal having a condition where inhibition of a cAMP-specific PDE is of therapeutic benefit, said method comprising administering to said mammal a therapeutically effective amount of a compound having a formula



or



wherein R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

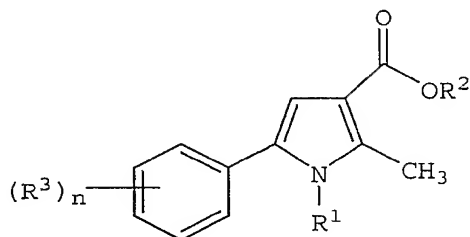
R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$ alkyl, NR^4R^5 , $C(=O)NR^5R^6$, $C(=O)$ Oalkyl, CO_2H , $OC(=O)$ alkyl, nitro, halo, alkylthio, SO_2 (alkyl), SO_3H , and haloalkyl;

- 127 -

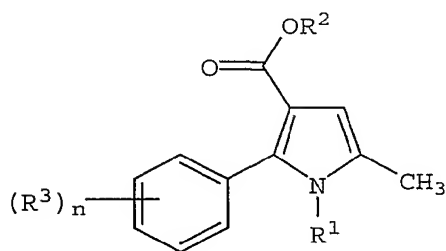
R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and n is 0 through 3.

- 128 -

20. A method of modulating cAMP levels in a mammal comprising administering to said mammal an effective amount of a compound of



or



wherein R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

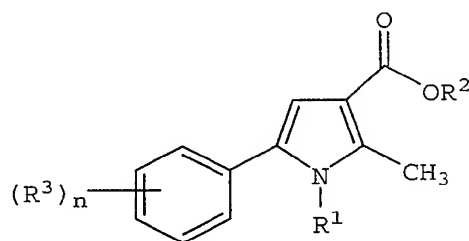
R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)alkyl$, NR^4R^5 , $C(=O)NR^5R^6$, $C(=O)Oalkyl$, CO_2H , $OC(=O)alkyl$, nitro, halo, alkylthio, $SO_2(alkyl)$, SO_3H , and haloalkyl;

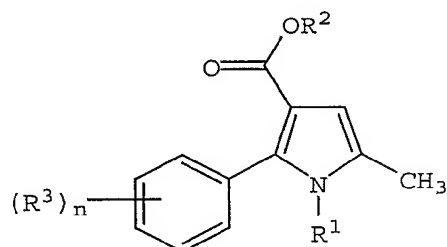
R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

21. A method of treating a mammal having a condition where inhibition of a cAMP-specific PDE is of a therapeutic benefit comprising administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a compound having a formula



or



wherein R¹ is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R² is alkyl or hydrogen;

R³ is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, hetero-aryl, aryloxyalkyl, aralkoxyalkyl, C(=O)alkyl, NR⁴R⁵, C(=O)NR⁵R⁶, C(=O)Oalkyl, CO₂H, OC(=O)alkyl, nitro, halo, alkylthio, SO₂(alkyl), SO₃H, and haloalkyl;

- 130 -

R⁴ and R⁵, independently, are hydrogen or alkyl, or R⁵ or R⁶ are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3;

a pharmaceutically acceptable carrier;

and,

optionally, a second anti-inflammatory therapeutic agent.

22. The method of claim 21 wherein the condition is an allergic disease, an autoimmune disease, an inflammatory disease, an arthritic disease, or dermatitis.

23. The method of claim 21 wherein the condition is rheumatoid arthritis, osteoarthritis, gouty arthritis, or spondylitis.

24. The method of claim 21 wherein the condition is thyroid-associated ophthalmopathy, Behcet disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, toxic shock syndrome, allergic conjunctivitis, vernal conjunctivitis, or eosinophilic granuloma.

25. The method of claim 21 wherein the condition is asthma, chronic bronchitis, allergic rhinitis, adult respiratory distress syndrome, chronic pulmonary inflammatory disease, chronic obstructive pulmonary disease, silicosis, or pulmonary sarcoidosis.

- 131 -

26. The method of claim 21 wherein the condition is reperfusion injury of the myocardium, brain or extremities as a brain or spinal cord injury due to minor trauma.

27. The method of claim 21 wherein the condition is a fibrosis, keloid formation, or scar tissue formation.

28. The method of claim 21 wherein the condition is systemic lupus erythematosus, a transplant rejection disorder, a graft vs. host reaction, or an allograft rejection.

29. The method of claim 21 wherein the condition is chronic glomerulonephritis, an inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

30. The method of claim 21 wherein the condition is proliferative lymphocytic disease or a leukemia.

31. The method of claim 21 wherein the condition is an inflammatory dermatosis, atopic dermatitis, psoriasis, or urticaria.

- 132 -

32. The method of claim 21 wherein the condition is a cardiomyopathy, congestive heart failure, atherosclerosis, pyrexia, cachexia, cachexia secondary to infection of malignancy, cachexia secondary to acquired immune deficiency syndrome, ARC, cerebral malaria, osteoporosis, a bone resorption disease, fever and myalgias due to infection, diabetes insipidus, a central nervous system disorder, depression, multiinfarct dementia, an anxiety or stress response, cerebral ischemia, tardive dyskinesia, Parkinson disease, and premenstrual syndrome.

33. The method of claim 21 wherein the mammal exhibits a minimal emetic response.

34. The method of claim 21 wherein the mammal is free of an emetic response.

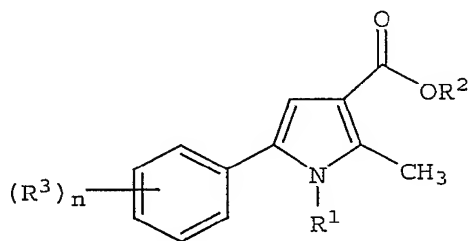
35. The method of claim 21 wherein the mammal exhibits minimal adverse central nervous system side effects.

36. The method of claim 21 wherein the mammal is free of adverse central nervous system side effects.

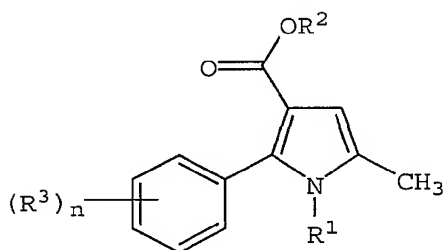
37. The method of claim 21 wherein the second anti-inflammatory therapeutic agent is capable of targeting TNF α .

- 133 -

38. A method of reducing TNF levels in a mammal comprising administering to said mammal a therapeutically effective amount of a compound having a formula



or



wherein R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

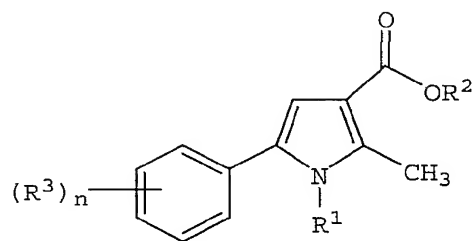
R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$ alkyl, NR^4R^5 , $C(=O)NR^5R^6$, $C(=O)$ oalkyl, CO_2H , $OC(=O)$ alkyl, nitro, halo, alkylthio, SO_2 (alkyl), SO_3H , and haloalkyl;

- 134 -

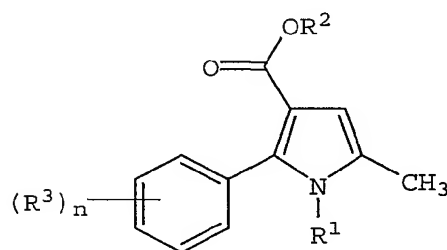
R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and n is 0 through 3.

- 135 -

39. A method of suppressing inflammatory cell activation in a mammal comprising administering to said mammal a therapeutically effective amount of a compound having a formula



OR



wherein R¹ is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R² is alkyl or hydrogen;

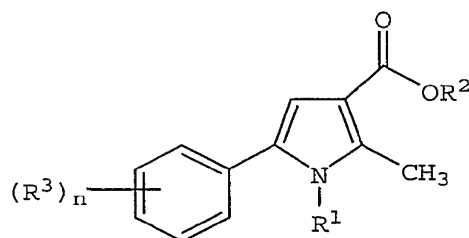
R³ is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, C(=O)alkyl, NR⁴R⁵, C(=O)NR⁵R⁶, C(=O)Oalkyl, CO₂H, OC(=O)alkyl, nitro, halo, alkylthio, SO₂(alkyl), SO₃H, and haloalkyl;

- 136 -

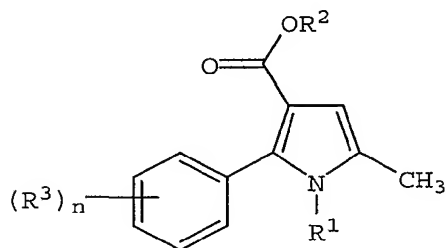
R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and n is 0 through 3

- 137 -

40. A method of inhibiting PDE4 function in a mammal comprising administering to said mammal a therapeutically effective amount of a compound



or



wherein R¹ is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R² is alkyl or hydrogen;

R³ is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, C(=O)alkyl, NR⁴R⁵, C(=O)NR⁵R⁶, C(=O)Oalkyl, CO₂H, OC(=O)alkyl, nitro, halo, alkylthio, SO₂(alkyl), SO₃H, and haloalkyl;

- 138 -

R^4 and R^5 , independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and n is 0 through 3.

1 / 1

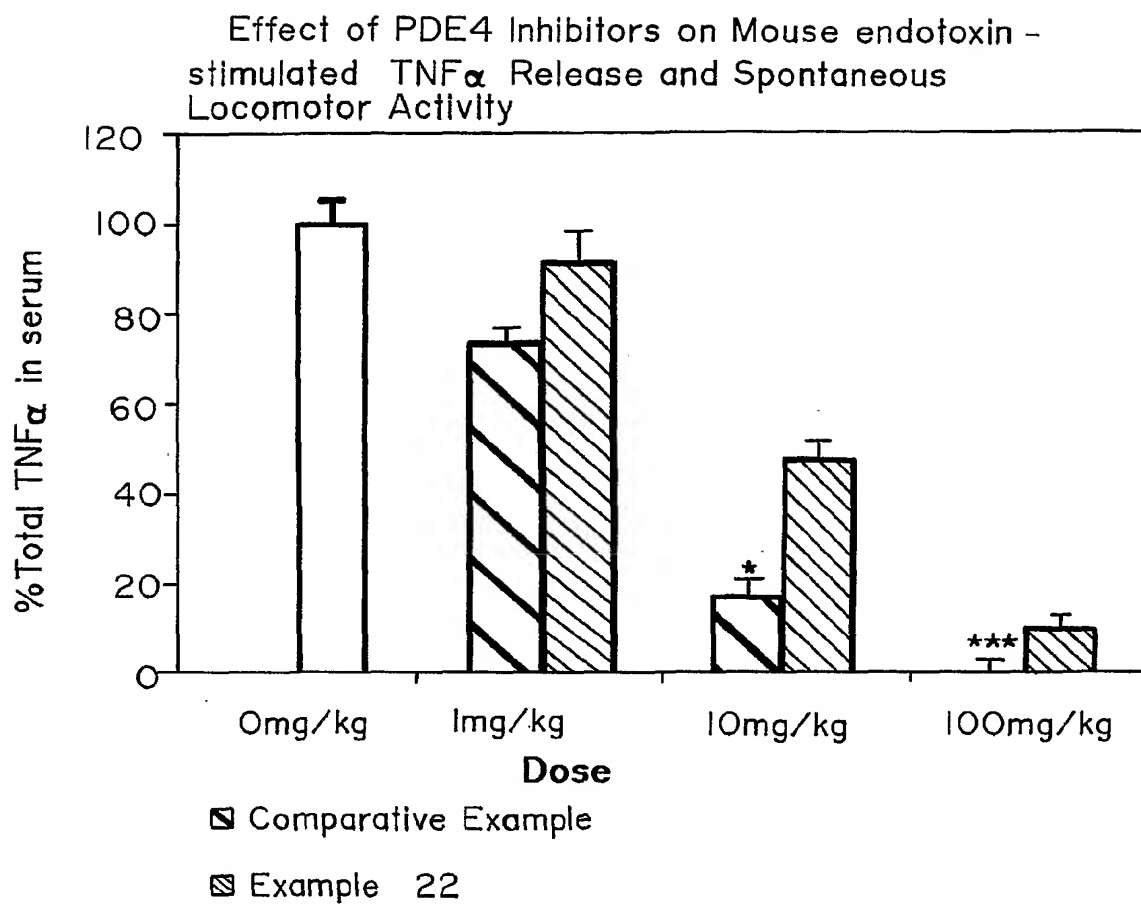


FIG. 1

INTERNATIONAL SEARCH REPORT

Intern: Application No

PCT/US 00/28496

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D207/34 A61K31/40 C07D401/04 A61P29/00 A61K31/402

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 799 823 A (SANKYO CO., LTD., JAPAN) 8 October 1997 (1997-10-08) see formula (Ia-1), page 32 page 89, line 23 - line 54 ---	1-5
X	CHEMICAL ABSTRACTS, vol. 97, no. 9, 30 August 1982 (1982-08-30) Columbus, Ohio, US; abstract no. 72206, GRINEV, A. N. ET AL: "Synthesis and biological activity of 4-oxoindeno'1,2-b!pyrrole derivative" XP002156580 abstract & KHIM.-FARM. ZH. (1982), 16(4), 433-8 , --- -/--	1-5

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 January 2001

Date of mailing of the international search report

47.03.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Schmid, J-C

INTERNATIONAL SEARCH REPORT

Internat. Application No

PCT/US 00/28496

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DEMIRAYAK, S. ET AL: "Synthesis of some 4-pyrrolylphenylthiazole derivatives and investigation of their antimicrobial activities" ACTA PHARM. TURC. (1997), 39(3), 133-136 , XP000945239 see compound 5d ---	1,2,5,6
X	KEATING, THOMAS A. ET AL: "Postcondensation Modifications of Ugi Four-Component Condensation Products: 1-Isocyanocyclohexene as a Convertible Isocyanide. Mechanism of Conversion, Synthesis of Diverse Structures, and Demonstration of Resin Capture" J. AM. CHEM. SOC. (1996), 118(11), 2574-83, XP002058962 see compounds 36 and 37 page 2578 ---	1-3,5,6
X	PORRETTA, GIULIO CESARE ET AL: "Research antibacterial and antifungal agents. XII - Synthesis and antimicrobial activity of some Mannich bases of diarylpyrroles" FARMACO (1995), 50(9), 617-23, XP000945182 see compound 9 page 619 ---	1-3,5
X	BACHECHI, FIORELLA ET AL: "A 1,5-diarylpyrrole derivative" ACTA CRYSTALLOGR., SECT. C: CRYST. STRUCT. COMMUN. (1995), C51(7), 1431- , XP000945267 see compound 2 ---	1-3,5
X	ANTONUCCI, T. ET AL: "Characterization of the antiviral activity of highly substituted pyrroles a novel class of non-nucleoside HIV-1 reverse transcriptase inhibitor" ANTIVIRAL CHEM. CHEMOTHER. (1995), 6(2), 98-108 , XP000945183 see compound 46 page 101 --- -/--	1-5,18

INTERNATIONAL SEARCH REPORT

Intern: | Application No

PCT/US 00/28496

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ALMERICO, ANNA MARIA ET AL: "Polycondensed nitrogen heterocycles. Part 26. Aminopyrrolo'1,2- f!phenanthridines by decomposition and cyclization of 2-aryl-1-(3- azidophenyl)pyrroles" HETEROCYCLES (1994), 37(3), 1549-59 , XP000945175 page 1551 -page 1552 ---	1-7
X	CERRETO, FELICE ET AL: "Synthesis and antimicrobial activity of some 1,5-diaryl-2-methyl-3- carbethoxy-4-(4-methylpiperazin-1-ylmethyl)pyrroles and some 1,5-diaryl-2-methyl-3,4-di(4-methylpiperaz in-1-ylmethyl)pyrroles" FARMACO (1993), 48(12), 1735-1746 , XP000945229 see formula 13 page 1738 ---	1-6
X	ALMERICO, ANNA MARIA ET AL: "Polycondensed nitrogen heterocycles. Part 25. Aminopyrrolo'1,2-f!- phenanthridines by decomposition of 2-(3-azidophenyl)-1-arylpyrroles" J. HETEROCYCL. CHEM. (1994), 31(1), 193-8, XP000945177 the whole document ---	1-5
X	SCALZO, MARCELLO ET AL: "Studies on anti-Candida agents with a pyrrole moiety. Synthesis and microbiological activity of some '(1-alkyl),(1-aryl) and (1-benzyl)-5-aryl-3-carboxamido-2-methyl!p yrrole derivatives" FARMACO (1992), 47(7-8), 1047-53 , XP000945192 see formula 1 and compounds 15-23 page 1048 -page 1049 ---	1-6
X	PORRETTA, GIULIO CESARE ET AL: "Chemotherapeutic agents with an imidazole moiety. III. Synthesis and microbiological activity of new 1,4-diarylimidazole and 1-(imidazol-4-yl)-4-(pyrrol-1-yl)benzenes" FARMACO (1991), 46(7-8), 913-24 , XP000945194 see compounds 6a-e page 915 --- -/--	1-3,5,18

INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/US 00/28496

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>COLOTTA, VITTORIA ET AL: "Tricyclic heteroaromatic systems. Synthesis of 1,3 and 1,2 disubstituted '1!benzopyrano'4,3-b!pyrrol-4-ones and structure-activity relationships a benzodiazepine receptor ligands" FARMACO (1991), 46(10), 1139-54 , XP000945195 see compounds 63-76 page 1148</p> <p>---</p>	1-5
X	<p>TEXIER, F. ET AL: "Regioselectivity in 1,3-dipolar cycloaddition of munchnones with two electrophilic alkynes. Attempted rationalization" BULL. SOC. CHIM. FR. (1991), (NOV.-DEC.), 962-7 , XP000945368 see compound 6 page 963</p> <p>---</p>	1-6
X	<p>PORRETTA, G. C. ET AL: "Research on substances with antibacterial and antifungal activities. Not VII. Synthesis and microbiological activity of new derivatives of 1,5-diarylpyrrole" FARMACO (1989), 44(1), 65-76 , XP000945349 see tabella (III) page 68</p> <p>---</p>	1-5
X	<p>PORRETTA, G. C. ET AL: "Research on substances with antibacterial and antifungal activities. Note VI. Synthesis and microbiological activity of new derivatives of 1,5-diarylpyrrole and 1,4-pyrrolophenylene" FARMACO (1989), 44(1), 51-63 , XP000945348 the whole document</p> <p>---</p>	1-5
X	<p>PETRUSO, S. ET AL: "Oxidative halogenation of substituted pyrroles with copper(II). Part II. Bromination of some ethyl 3-pyrrolecarboxylates and corresponding acids" J. HETEROCYCL. CHEM. (1990), 27(5), 1277-80 , XP000945179 see table 1</p> <p>---</p> <p style="text-align: center;">-/--</p>	1-8

INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/US 00/28496

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HE, MEIYU ET AL: "Cyclization of 1,2,3,5-polysubstituted pyrroles in electron impact" ORG. MASS SPECTROM. (1989), 24(4), 271-5 , XP000945280 the whole document ---	1-5
X	SCALZO, MARCELLO ET AL: "Synthesis and microbiological activity of new 1,5-diarylpyrroles" EUR. J. MED. CHEM. (1988), 23(6), 587-91 , XP000945383 the whole document ---	1-5
X	SCALZO, M. ET AL: "Compounds with antibacterial and antifungal activity. Part IV. Synthesis and microbiological activity of new 1,5-diarylpyrrole derivatives" FARMACO, ED. SCI. (1988), 43(9), 665-76 , XP000945201 the whole document ---	1-8
X	SCALZO, M. ET AL: "Compounds with antibacterial and antifungal activity. Part V. Synthesis and microbiological activity of new 1,5-diarylpyrrole derivatives" FARMACO, ED. SCI. (1988), 43(9), 677-91 , XP000945203 the whole document ---	1-5
X	CHEMICAL ABSTRACTS, vol. 109, no. 11, 12 September 1988 (1988-09-12) Columbus, Ohio, US; abstract no. 92698, GRINEV, A. N. ET AL: "Intramolecular cyclization of 5-(o-carboxyphenyl)pyrroles" XP002156581 abstract & KHIM. GETEROTSIKL. SOEDIN. (1987), (10), 1333-5 , ---	1-5
X	CIRRINCIONE, GIROLAMO ET AL: "Polycondensed nitrogen heterocycles. Part 20. Reactivity of diazotized 1-(2-aminophenyl)pyrroles. Pyrrolo'1,2-f!phenanthridines" HETEROCYCLES (1986), 24(12), 3403-10 , XP000945339 see compounds 2b, 6b --- -/--	1-8

INTERNATIONAL SEARCH REPORT

Intern Application No

PCT/US 00/28496

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PORRETTA, G. C. ET AL: "Pyrrolnitrin analogs. XI. Synthesis and microbiological activity of new 1,4- and 1,5-diaryl-pyrroles" FARMACO, ED. SCI. (1985), 40(8), 589-607 , XP000945205 see formula XIV and XV page 592 ---	1-5
X	CHEMICAL ABSTRACTS, vol. 102, no. 9, 4 March 1985 (1985-03-04) Columbus, Ohio, US; abstract no. 78005, HUA, WENTING: "Vicinal group effect in Knorr-Paal condensation reaction" XP002156582 abstract & HUAXUE TONGBAO (1984), (10), 31-4, 7 , ---	1-5
X	FILACCHIONI, G. ET AL: "Pyrrolnitrin analogs. VIII. Synthesis and antibacterial activity of new 1,4- and 1,5-diarylpyrroles" FARMACO, ED. SCI. (1983), 38(9), 630-9 , XP000945207 the whole document ---	1-5
X	FILACCHIONI, G. ET AL: "Pyrrolnitrin analogs. VII. Ethyl 1,4- and 1,5-diaryl-2-methylpyrrole-3- carboxylates from phenacylarylamines and ethyl acetoacetate" FARMACO, ED. SCI. (1983), 38(9), 619-29 , XP000945206 the whole document ---	1-5
X	CHEMICAL ABSTRACTS, vol. 97, no. 21, 22 November 1982 (1982-11-22) Columbus, Ohio, US; abstract no. 182142, HUA, WENTING ET AL: "Synthesis of sterically crowded polysubstituted pyrroles. (II)" XP002156583 abstract & HUAXUE TONGBAO (1982), (5), 281-4, 314 , --- -/--	1-5

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/28496

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATTOLO, GAETANO ET AL: "Polycondensed heterocycles. Part XI. 1,3-Disubstituted 2-methylpyrrolo'3,2-c!cinnoline and 2-acetyl-3-methylpyrrolo'1,2-c!benzo'1,2,3!triazine" HETEROCYCLES (1982), 19(4), 681-6 , XP000973101 see compound 1	1-5
X	FILACCHIONI, G. ET AL: "Pyrrolnitrin analogs. VI. Unusual pathway in the Knorr synthesis between N-(4-nitrophenacyl)dimethylanilines and ethyl acetoacetate" FARMACO, ED. SCI. (1979), 34(11), 987-1004, XP000945210 the whole document	1-5
X	CHEMICAL ABSTRACTS, vol. 88, no. 11, 13 March 1978 (1978-03-13) Columbus, Ohio, US; abstract no. 74256, KEJHA, J. ET AL: "Preparation and evaluation of the antiinflammatory effectiveness of substituted 4-arylpyrrole-3-carboxylic acids and 5-phenyl-2-methyl-3-carboxypyrrole-4-aliphatic acids" XP002156584 abstract & CESK. FARM. (1977), 26(6), 220-6 ,	1-6
X	CHEMICAL ABSTRACTS, vol. 63, no. 10, 8 November 1965 (1965-11-08) Columbus, Ohio, US; abstract no. 13189b, MCKINNON, DAVID M: "Feist synthesis of pyrrole-3-carboxylic esters" XP002156585 abstract & CAN. J. CHEM. (1965)43(9), 2628-31,	1-6

-/--

INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/US 00/28496

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 63, no. 12, 6 December 1965 (1965-12-06) Columbus, Ohio, US; abstract no. 16289g, SCHILFFARTH, KARLCHRISTIAN ET AL.: "aryl-substituted 1,1'-bipyrryls and their dissociation into radicals" XP002156586 abstract & CHEM. BER. (1965), 98(10), 3124-32,	1-6
X	----- CHEMICAL ABSTRACTS, vol. 55, no. 22, 30 October 1961 (1961-10-30) Columbus, Ohio, US; abstract no. 22337, FATUTTA, SILVANA: "ethyl alpha-(p-phenylphenacyl) acetoacetate and related heterocyclic derivatives" XP002156587 abstract & ANN. CHIM. (1961), 51, 252-65,	1-5
A	----- US 5 665 754 A (FELDMAN PAUL LAWRENCE ET AL) 9 September 1997 (1997-09-09) cited in the application the whole document	1-40
A	----- WO 92 19594 A (SMITHKLINE BEECHAM CORP) 12 November 1992 (1992-11-12) the whole document -----	1-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/28496

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19-40 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.
2. ☒ Claims Nos.: 1,5
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,5

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claim(s) may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible. Consequently, the search has been restricted to the compounds of claim 2

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/28496

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0799823 A	08-10-1997	AU 710380 B	16-09-1999
		AU 1665397 A	09-10-1997
		CA 2201812 A	05-10-1997
		CN 1168372 A	24-12-1997
		CZ 9701035 A	15-10-1997
		HU 9700709 A	28-05-1999
		JP 3034819 B	17-04-2000
		JP 9323971 A	16-12-1997
		NO 971564 A	06-10-1997
		NZ 314508 A	29-06-1999
		RU 2125044 C	20-01-1999
		US 5908858 A	01-06-1999
US 5665754 A	09-09-1997	AT 194593 T	15-07-2000
		AU 685170 B	15-01-1998
		AU 7839694 A	10-04-1995
		CA 2171448 A	30-03-1995
		DE 69425267 D	17-08-2000
		DE 69425267 T	18-01-2001
		EP 0720600 A	10-07-1996
		ES 2149888 T	16-11-2000
		JP 9502979 T	25-03-1997
		NZ 274381 A	26-02-1998
		WO 9508534 A	30-03-1995
WO 9219594 A	12-11-1992	AU 1917092 A	21-12-1992
		CA 2102106 A	03-11-1992
		CN 1067244 A	23-12-1992
		EP 0584208 A	02-03-1994
		IE 921376 A	04-11-1992
		JP 6507405 T	25-08-1994
		MX 9202090 A	01-11-1992
		PT 100441 A	30-09-1993
		ZA 9203210 A	31-03-1993